“Screening of tomato germplasm and functional characterization of candidate purple acid phosphatase genes under phosphate deficiency”

A thesis submitted to University of Hyderabad for the award of
Doctor of Philosophy (Ph.D.)
by

Rajat Srivastava
Reg. No. 17LPPH13

INSTITUTE OF EMINENCE

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(A Central University established in 1974 by act of parliament)

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Hyderabad– 500 046, INDIA

DECLARATION

I, **Rajat Srivastava,** hereby declare that this thesis entitled ***"Screening of tomato germplasm and functional characterization of candidate purple acid phosphatase genes under phosphate deficiency"*** submitted by me is based on the results of the work done under the guidance and supervision of **Dr. Rahul Kumar** at the Department of Plant Sciences, School of Life Sciences, University of Hyderabad. The work presented in this thesis is original and plagiarism free. I also declare that no part or in full of this thesis has been submitted previously to this University or any other University or Institution for the award of any degree or diploma.

Rajat

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CERTIFICATE

This is to certify that the thesis entitled ***"Screening of tomato germplasm and functional characterization of candidate purple acid phosphatase genes under phosphate deficiency"*** submitted by **Rajat Srivastava** bearing registration number **17LPPH13** in partial fulfilment of the requirement for the award of Doctor of Philosophy in the School of Life Sciences is a bonafide work carried out by him under my supervision and guidance.

The thesis is free from plagiarism and has not been submitted previously in part or in fully to this or any other university or institution for the award of any degree or diploma.

Part of the thesis have been published in the following publications:

1. **Srivastava, R.**, Akash., Parida, A.P., Chauhan, P.K., Kumar, R., 2020. Identification, structure analysis, and transcript profiling of purple acid phosphatases under Pi deficiency in tomato (*Solanum lycopersicum*) and its wild relatives. Int. J. Biol. Macromol. 165, 2253-2266. DOI: 10.1016/j.ijbiomac.2020.10.080.
2. Akash., **Rajat Srivastava** and Kumar, R. (2021). VIGS-based gene silencing for assessing mineral nutrient acquisition. Plant gene silencing. Methods in Molecular Biology, 2nd edition vol. 2408. DOI: 10.1007/978-1-0716-1875-2_11.

Presented the poster in the following conferences:

1. **Rajat Srivastava,** Gayathri A.G. and Rahul Kumar, presented a psoter **“Understanding the SPX-PHR controlled mycorrhizal colonization in tomato roots using Virus-induced gene silencing approach”** at International Conference on Virus Evolution, Infection and Disease Control (ICVEIDC-2022) bydepartment of Biotechnology & Bioinformatics, School of Life Sciences, University of Hyderabad on 15th-17th December 2022, India-500046.
2. **Rajat Srivastava,** Gayathri A.G. and Rahul Kumar, Presented poster on **“Understanding the genetic regulatory mechanism controlling PUE and PAE upon mycorrhizal colonization in tomato”** in 12 th India-Japan Science and Technology. International conference on Frontier areas of science and technology (ICFAST-12022) held at University of Hyderabad, Hyderabad 500046, INDIA during september 09-10-2022.
3. **Rajat Srivastava** and Rahul Kumar, presented poster on “**Transcriptomics and functional genomics for improving phosphate use efficiency in tomato (Solanum lycopersicum)”** at National conference on Integrative Plant Biochemistry and Biotechnology, 08-09 November 2019, organized by Society for Plant Biochemistry and Biotechnology, New Delhi and IICAR-IRR, Hyderabad India-500046.
4. Akash, **Rajat Srivastava** and Rahul Kumar entitled “**Understanding the molecular circuitry underlying low phosphorous response in tomato”** in BioQuest-2017 held on October 12 - 132017 at School of Life Sciences, University of Hyderabad, Hyderabad, India-500046.

Further, the student has passed the following courses towards the fulfillment of the coursework requirement for Ph. D.

Course code	Name	Credits	Pass/Fail
PL 801	Research Methodology/Analytical techniques	4	Pass
PL 802	Research ethics, Biosafety, Data analysis, and Biostatistics	4	Pass
PL 803	Scientific Writing and Research Proposal	4	Pass

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Rajat srivastava…....

Table of Contents

Abbreviation

µL=Micro liter.
µm=Micro meter.
Ab=Antibiotic.
B=Breaker.
bp=Base pair.
Ca=Calcium.
cm=Centimeter.
DW=dry weight.
Fe=Iron.
gm=Gram.
kb=kilobase.
KO=Knockout.
Mg=Magnesium.
min=Minute.
ml=Milli liter.
mm=Milli meter.
N=Nitrogen.
no=Number.
O/N=Overnight.
PR=Pusa ruby.
Rif=Rifampicin.
sec=Second.
Zn=Zinc.
µM=Micro Molar.
C=Degree Celsius.
Col-0=Columbia-0.
Hyg=Hygromycin.
Kan=Kanamycin.
nM=Nano Molar.
Abs=Absorbance.

APase=Acid Phosphatase.
CDS=Coding sequence.
DAT=Day after treatment.
FW=Fresh weight.
GUS=β-glucuronidase.
HM=Homozygous.
Liq. N_2=Liquid Nitrogen.
LP=Low Phosphate.
MCT=Microcentrifuge tube.
N2=Liquid Nitrogen.
NaCl=Sodium Chloride.
NaOH=Sodium Hydroxide.
NBT=Nitro blue tetrazolium.
ns=non-significance.
PDS=Phytoene desaturase.
Pi=Inorganic phosphate.
PM=Plasma membrane.
RH=Relative humidity.
WP=Whole Plants.
DAB=3,3′-Diaminobenzidine.
DDW=Double distilled water.
HP=High Phosphate.
SAP=Secretary Acid Phosphatase.
OX=Over-expression
AVai=Arka Vaibhav
AV=Arka Vikas.
PR=Pusa Ruby.
ROS=Reactive oxygen species.

BCIP=5-Bromo-4-chloro-3-indolyl phosphate.
CRISPR=Clustered regularly interspaced short palindromic repeats.
P1BS=Phosphate Starvation Response1 binding site.
pNPP=Para-Nitrophenyl phosphate.
PSI=Phosphate Starvation Inducible.
PSR=Phosphate Starvation Response.
qPCR=Quantitative Polymerase Chain reaction.
VIGS=Virus-induced gene silencing.
pSlPAP15=SlPAP15 promoter construct.
PUE=Pi use efficiency.
PAE=Phosphorus acquisition efficiency.
DAT=Days after treatment.
TRV=Tobacco rattle virus.
CaMV=Cauliflower mosaic virus.
MCS=Multiple cloning site.
EV=Empty vector.
SPX= SYG1 (suppressor of yeast gpa1), Pho81 (CDK inhibitor in yeast PHO pathway), and XPR1 (xenotropic and polytropic retrovirus receptor).
MUG= 4-methylumbelliferyl-beta-D-glucuronide
PEP=Phosphoenolpyruvate.
IHP=Inositol-hexaphosphate.
AMF=Arbuscular mycorrhizal fungi.
MGR=Mycorrhizal growth response.

GO=Gene ontology. TFs=Transcription factors. mM= Milli molar	BCIP=5-bromo-4-chloro-3-Indolyl-phosphate. PHR1=Phosphate starvation response 1. Chr=Chromosome. P1BS=PHR1 binding sequence.	MER=Mycorrhizae effectivity ratio. SYMRK=Symbiotic receptor like kinase. GH=Gretchen hagen. Spe=Solanum pennelli. ICM=Integral component of membrane.

Introduction

Phosphate is a vital macronutrient which is required by plant and all the living organisms for their growth expansion and development. Phosphate (P), is the second most limiting plant nutrient after Nitrogen. It is also the essential component of nucleic acid, cell membrane and ATP. Nucleic acid such as DNA and RNA are the important genetic component of cell which carries the information to be translated to proteins for proper functioning of living things. While in membrane it maintains the cell integrity via protection from different biotic and abiotic stress. Being an important part of plant various metabolic reactions, signal transduction, energy transfer reaction, photosynthesis and respiration, its limitation causes a major constraint for plant productivity in natural environment. Therefore, a holistic approach is needed to combine Pi-use efficient germplasm with the targeted fertilization, agronomically superior fertilizer formulations for improved growth of plant via use of organisms such as PSB (phosphate solubilizing bacteria), AMF (arbuscular mycorrhizae fungi). While use of Omics approach was used to identify and target candidate genes having role in improving PAE (phosphate acquisition efficiency) and PUE (phosphate use efficiency) have provided a new dimension in betterment of plant overall growth.

1. Soil Phosphorus (P) Status: Assessing and Managing Nutrient Levels

Soils around the globe vary greatly from very high to very low in terms of the spatial distribution. MacDonald et al. (2011) has suggested that the soils in the majority of global agrosystems (~ 70 %) is rich in P [1]. Despite this, only a small percentage of soil P is often available to plants. A large fraction, amounting up to 80 %, of total soil P is available in its organic form, especially as phytic acid (inositol hexaphosphate) [2,3]. The Pi absorbed by roots from the soils are mainly available in its orthophosphate forms such as $H_2PO_4^-$ or HPO_4^{-2} [4]. There are two primary P sources in the soils, (i) the native P and (ii) externally applied P, as organic or inorganic complexes. The bioavailability of Pi is generally limited in the soils due to its rapid fixation with cations such as Ca, Al, and Fe [5][6]. These insoluble complexes together with the organic P pools (mainly the decomposed parts of the living organisms) make it poorly mobile in the soils [6]. To cope with Pi limitations, plants have acquired several adaptive strategies, collectively called Pi starvation responses (PSRs). These adaptive measures are employed to improve Pi uptake and internal mobilization for its better access to plants [7–9]. According to an estimation, about half of the agricultural soils worldwide are deficient in P [10]. Such deficiency in soils is either because of inadequate P replenishment into agrosystems (more likely in developing countries with limited access to fertilizers) or due to its fixation, which renders soil P unavailable to plants [11]. Thus, soil P may be copious in the soils of some regions, but its unrestricted availability to plants is not guaranteed. Therefore, chemical Pi-fertilizers are routinely applied to replenish the inorganic and bioavailable P deficiency in agricultural systems [12]. The bioavailability of P is strongly affected by soil pH as mildly acidic soil pH favours its maximum solubility and plant availability. The formation of iron (Fe) and aluminum (Al) phosphate minerals such as strengite ($FePO_4.2H_2O$) and variscite ($AIPO_4.2H_2O$), respectively, reduce its bioavailability in strongly acidic soil [13]. Likewise, in the alkaline pH (around 8), soil Pi reacts with calcium (Ca) and form insoluble Ca-Pi complexes [14–16]

2. World Phosphate Rock Reserve Status: Availability and Global Distribution

P-reserves are a non-renewable resource that is asymmetrically distributed globally. Three countries, including Morocco and Western Sahara (71 %), China (4.8 %), and Algeria (3 %), together hold most of the global natural rock phosphorous (RP) reserves (Survey USG 2020). The majority of the developing countries lack large natural indigenous P-reserves and meet their demand by importing RPs or finished fertilizers. In past decades, the production and demand for Pi-fertilizers has steadily increased **(Fig. 1).** Numerous statistical analyses predict that the demand for Pi would further increase and reach its peak by 2030 [17]. Although there is no consensus on the exact time of the complete depletion of RPs, P-reserves are anticipated to exhaust in the next few hundred years [18–20]. Thus, a potential P crisis is imminent in the recent future that would challenge the sustainability of agriculture.

3. Strategies for phosphate Acquisition and Utilization

Plant response to low Pi involve series of changes at morpho-physiological and molecular level. Developmental changes include primarily the modification of RSA (root system architecture) such as the development of proteoid roots and cluster roots, hence providing the environment for uptake of Pi from rhizosphere. PSRs are classified into local and systemic re

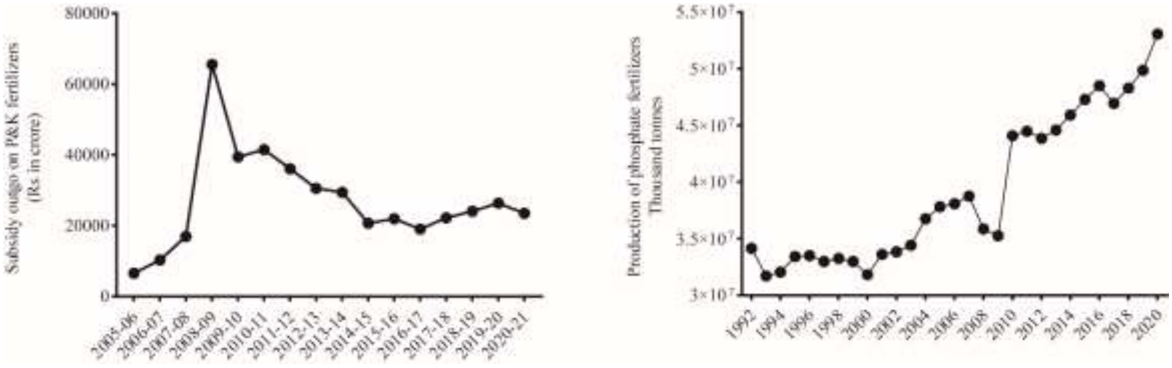


Fig 1: *Global production of Pi fertilizer and annual subsidy outgo for P&K fertilizer. Data was obtained from FAO website. (Srivastava et al., 2021)*

sponses [21,22]. The profound structural modifications in plants under Pi deficiency unarguably occur in the root system architecture (RSA), a localized PSR [23,24]. Modifications in root system are triggered by local alterations in external Pi and sensed by the root tip under depleted Pi conditions [25]. As plants assimilate Pi primarily via roots, the reprogramming of RSA under Pi deficiency is advantageous to improve nutrients foraging. Altered primary root length, increased lateral roots, and longer and denser root hairs are some of the well-studied morphological changes observed in the roots of Pi-deficient plants [26–28]. Formation of cluster roots and a decrease of root angles to a shallower position also occur in Pi-deprived plants [29–31]. The development and functioning of cluster roots in white lupin and the Proteaceae family members is a well-studied strategy by plants to maximize Pi uptake from its enriched patches in the soils [32–35]. Since cluster roots in Proteaceae members are more prominent in the top layers of the soils, secretion of organic acids strategy is preferred over the P scavenging through root extension to mobilize sparingly available Pi [36]. The decreased growth angle of basal roots is also considered an effective strategy to maximize Pi-acquisition from the top layers of the soil in several plant species [37,38]. Although RSA modification is a common adaptive response in plants subjected to Pi deficiency, comparison of the low Pi-induced RSA in Arabidopsis and several other crop germplasms indicates that such response is genetically determined and is highly species-specific [39–43].

4. Metabolic adaptations to increasing P availability in rhizosphere

Secretion of organic acids, protons, phosphatases, or ribonucleases by roots in the rhizosphere is an efficient mechanism adopted by plants to improve Pi-acquisition from the organic P and insoluble-Pi mineral complexes in the soils [11,44,45]**.** It is a localized PSR that helps mobilize and enhance the bioavailability of the available Pi in the soils [46]. Releasing protons or organic acids by Pi-deficient roots causes acidification of the rhizosphere. Consequent mineralization of insoluble Pi-complexes or organic P compounds results in the release of Pi in the rhizosphere. Root exudation of carboxylates such as citrate, malate, and oxalate mobilizes Pi from Al, Fe, or Ca complexes through chelation and ligand exchanges in the rhizosphere [47]. In this context, the role of malate not only in the mineralization of soil bound P but also in controlling RSA under Pi depletion is well studied. A low Pi supply promoted organic acids exudation and simultaneously enhanced Fe absorption during its deficiency in apples. Another critical strategy to improve Pi-mineralization is the secretion of phosphatases or phytases by Pi-starved roots. Their release mobilizes Pi from the organic phosphate complexes through enzyme catalyzed hydrolysis [47]. A large body of evidence has demonstrated the induction of numerous phosphatases encoding genes in many plant species under Pi deficiency [48–52]. Several purple acid phosphatases (PAPs) and haloacid halogenases have been implicated to increase PUE and PAE in plants.

5. Optimizing phosphate balance: A Comprehensive Approach to Plant Nutrient Management

5.1 Role of Purple acid phosphatase: Empowering PAE and PUE

Purple acid phosphatases (PAPs; EC 3.1.3.2) are members of metallo-phosphoesterase family of binuclear metal containing acid hydrolases. These enzymes are ubiquitously present in animals, plants, bacteria, and fungi. PAPs hydrolyze a wide range of phosphomonoester and amide substrates and develop a peculiar purple or pink color in solution [53–55]. These enzymes were first discovered in mammals. Since then, several PAP isoforms have also been purified from plant tissues [51,56,57]. The binuclear metal center of these enzymes in its active form consists of either Fe (III)-Me (II) where Me stands for iron, zinc, or manganese [58]. Between the two, while only the Fe(III) is a chromophoric metal ion, but both metals are crucial for the function of the enzymes, and they need to have different oxidation states [59–61]. Mutations in these respective metal ion binding sites often result in the loss of enzymatic activity [62]. Usually, seven invariant metal binding amino acid residues (DXG/GDXXY/GNH(D/E)/VXXH/GHXH; bold letters indicate metal-ligating residues), are found in the five conserved motifs present at the carboxyl end of PAP members. These residues are required for active bimetallic centers and their activity. For instance, the five conserved blocks of amino acid residues are involved in Fe(III)–

Fe(II) binding in mammalian PAPs and, Fe(III)–Fe(II), Fe (III)–Zn(II), or Fe(III)–Mg(II) metal ion binding in plants [63,64]. Owing to their physiological roles in P nutrition and intracellular homeostasis, the full complement of PAPs have been identified in many plants, including Arabidopsis (29), rice (26), soybean (35), chickpea (25), Jatropha, and *Camellia sinensis* [57,64–67].However, the precise physiological role(s) and preferred substrate(s) of many PAPs remain obscure in plants and warrant further investigations [68]. Based on their molecular weight, plant PAPs are usually grouped into high molecular weight or large PAPs and low-molecular-weight or small PAPs [69,70]. Large PAPs are oligomeric proteins with a polypeptide of about 55 kDa and two domains, whereas small PAPs are monomeric proteins with a polypeptide of 35 kDa and one domain. While large PAPs are homologous to fungi and mycobacteria enzymes, small PAPs are closely related to mammalian homologs [53,63,69]. Due to their activation under P deficiency, plant PAPs are mainly implicated in phosphate (Pi) acquisition and utilization. However, their other potential roles in the generation of reactive oxygen species [71–73] , flower development [74], cell wall biosynthesis [75–77], defense response [78], and nitrogen and carbon metabolism [79] have also emerged. Biochemically, these enzymes have been studied for their catalytic roles during the hydrolysis of various phosphate complexes, including esters and an- hydrides in the pH range 4–7 [63,80]. While a considerable large number of plant PAPs tend to act on a wide range of substrates, a few phytate-preferential PAPs such as NtPAP, AtPAP15, AtPAP23, GmPhy, and OsPHY1 have also been identified. The major substrates for non-specific PAPs include ATP, phosphoenolpyruvate, and phosphoproteins [81][57][65][82–86]. PAPs can be dual targeted, as in the case of the *AtPAP26* gene, which encodes a protein with both intracellular and extracellular PAP activities [87]. Since the entire range of the functions of PAP members have not been deciphered to date, assigning specific roles to many uncharacterized PAPs and finding their preferred substrates remain a challenge. Nevertheless, a few PAPs have been manipulated in crops to improve phosphate acquisition efficiency. For example, overexpression of AtPAP15 improves Pi efficiency in soybean [88]. Similarly, overexpression of OsPAP21b and OsPAP26 improves phosphate acquisition, utilization, or remobilization in rice [51][89].

5.2 Intracellular Phosphorus Homeostasis: Balancing Pi Levels

Pi deficiency profoundly affects plants' carbon fixation, glycolysis, and respiration [90]. Reducing the ATP demand to recycle and optimize internal Pi use is another metabolic strategy to mitigate its deficiency by plants [91]. Activation of inorganic pyrophosphate-dependent bypass enzymes is crucial for the metabolic adaptations of plants under depleted cellular Pi [92]. Such changes facilitate the carbon flux for the enhanced synthesis of organic acids in the glycolytic pathway under chronic Pi limitation and help plant survival under depleted ATP levels [92]. Up-regulation of alternative oxidases in mitochondria also contributes to such adaptation by maintaining the mitochondrial citric acid cycle and electron transport chain with impaired ATP production, especially under long-term starvation [93,94]. During Pi starvation, enhanced sugar mobilization from shoot to roots occurs to support reprogramming of RSA [95,96]. Further, root sugar levels seem vital for the secretion and activity of root- associated acid phosphatase as a mutation in a sucrose transporter gene (SUC2) lowered such activity in mutant Arabidopsis seedlings and impacted Pi uptake [97]. Emerging evidence suggests that exogenous sucrose supply under Pi starvation conditions enhances the activation level of several Pi starvation inducible (PSI) genes in plants, highlighting the importance of sugar signaling to PSRs [98–101]. Remodeling of membrane lipids is another prominent alternate mechanism for Pi uptake under its acute deficiency. In such conditions, membrane phospholipids are converted into galactolipids. First, phospholipids, such as phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine are converted into diacylglycerol (DAG), which is then converted into galactolipids such as monogalactosyldiacylglycerol (MGDG) or digalactosyl diacylglycerol (DGDG). MGDG and DGDG synthetases catalyze these reactions [102][103][104]. A recent article published by Dissanayaka et al. (2021) has provided an in-depth overview of metabolic adaptations during PSRs in plants, including the mechanisms underlying phospholipid to galacto- and sulpholipid conversation under Pi starvation [45].

5.3 Alternative Phosphorus Sources and Efficient Recycling

Unearthing alternate Pi sources is critical to reducing over- reliance on the import of RPs. For example, phosphite (Phi), which has a higher solubility in water and is less prone to fixation in the soil, may be exploited as an alternate Pi-fertilizer [11]. Attempts have been made to develop bioengineered plants with the potential to utilize Phi as Pi-fertilizer [105]. Expression of phosphite oxidoreductase (ptxD) enzyme coding gene in Arabidopsis helped transgenic plants efficiently convert Phi to Pi after its uptake by the roots. These bioengineered plants only required 50 to 70 % of the original Pi input to achieve maximum productivity with Phi. Likewise, nuclear expression of *ptxD* in C. *reinhardtii* rendered transgenic lines the advantage of successfully metabolizing Phi as its Pi source [106]. Nonetheless, the natural inability of plants to metabolize Phi combined with high production cost and toxicity remains a bottleneck in its way to become a sustainable alternative to Pi-fertilizers. Further, due to its herbicidal properties, the use of Phi is banned in several European countries. Although the usage of Phi as an alternative Pi-fertilizer under the present scenario seems unrealistic, the development of Phi metabolizing plants which offers an alternative strategy to target both Pi nutrition with weed control cannot be neglected [11,107,108]. The amount of annually produced organic waste in big developing countries is enormous. Several available innovative technologies could be helpful for recycling and extracting Pi from urban, industrial, and agricultural waste. A few such technologies are already in place in Europe. For instance, sewage sludge ash is considered one of the best Pi sources across many European countries. Theoretically, it could meet 40-50 % of annually applied Pi-fertilizer input in agriculture in central Europe [109][110]. However, one of the biggest concerns about recovering Pi from municipal waste is its contamination with heavy metals and organic micropollutants. Therefore, modern techniques should be explored to recover Pi from sewage sludge with minimum contaminants. A cue in this context can be taken from a recently published article by Egle et al. (2016) [109]. The authors compared a total of 19 relevant Pi recovering technologies by considering their relationships with existing wastewater and sludge treatments. The outcome indicates that while clean and plant-available Pi is recoverable from municipal wastewater, the overall recovery remains poor. The present situation demands further technological innovations to improve the efficiency of the comprehensive Pi reclamation **(Fig. 2)**.

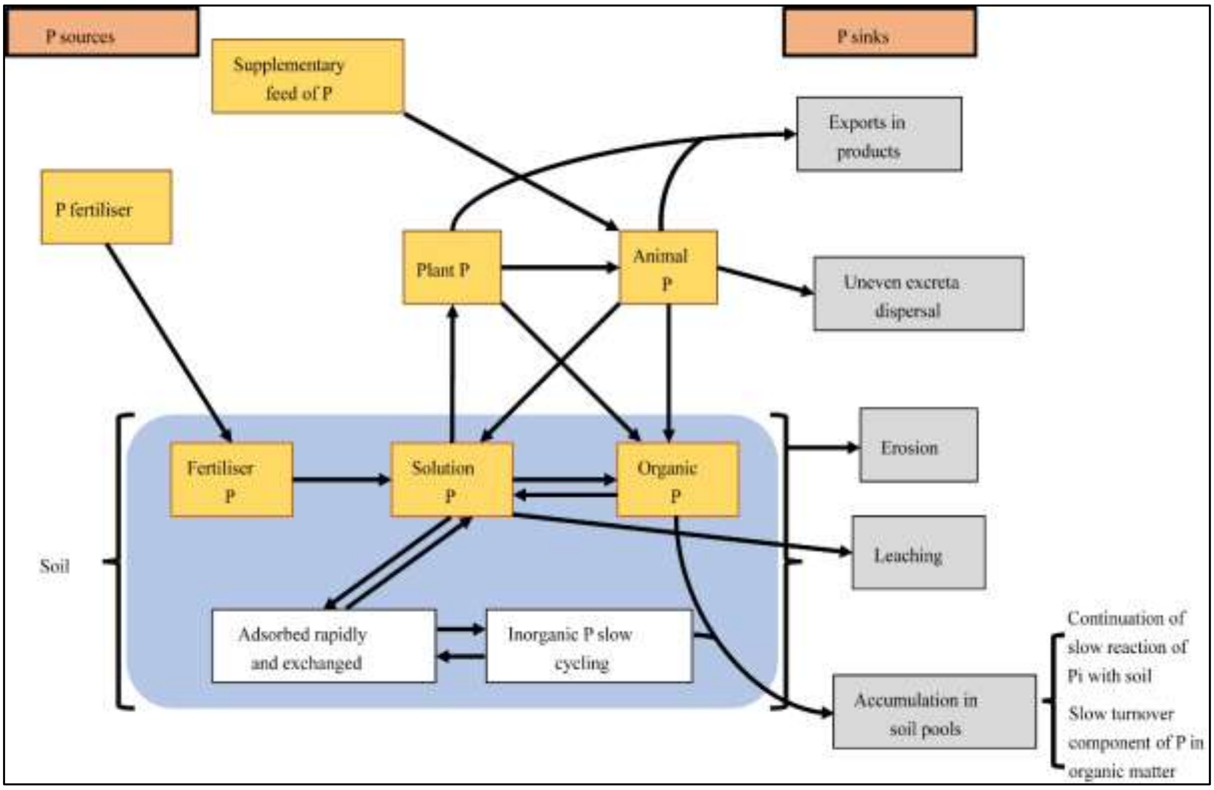


Fig 2: Schematic for the cycling of phosphorus in agriculture with emphasis on its utilization and acquisition from various sources. *Diagram illustrates interconnected components influencing phosphorus dynamics in agricultural systems. Soil erosion and leaching depict potential pathways for phosphorus displacement and nutrient loss. Excreta disposal represents the deposition of organic waste, serving as a phosphorus source. Organic P represents the presence of organic forms of phosphorus in the soil. P source encompasses various inputs like inorganic fertilizers, organic amendments, and recycled nutrients. P sink signifies the uptake and utilization of phosphorus by plants for growth. Supplementary feed of P represents the addition of supplemental phosphorus to meet plant nutrient requirements. Together, these components demonstrate the complex interplay between soil erosion, nutrient sources, sinks, and management strategies in phosphorus dynamics.*

6. Identification and development of P-use-efficient crops

As per the definition by Hammond and White (2008), PUE is defined as the amount of total biomass or yield produced per unit of absorbed P [95]. Plants are unable to assimilate majority of the applied Pi-fertilizers [111]. One of the effective measures that have been suggested to significantly bring down the scale of Pi-fertilizers input in agricultural systems is the identification of crop germplasm with better internal PUE and Pi-acquisition. The high PUE genotypes would efficiently mobilize residual soil P. Although PUE involves a complex set of plant traits, two basic mechanisms are central to it. First, the root's ability to reclaim residual Pi from the soil (also known as Pi- acquisition efficiency, PAE; or external PUE). Generally, crops germplasm with higher P scavenging capacity is recommended for soils particularly rich in organic P and insoluble Pi-complexes. Second, the efficiency of internal remobilization of Pi for sustainable biomass production (also known as the internal PUE). In the second scenario, crop genotypes which can give better yield in under-fertilized soils are recommended [112]. While identifying crops germplasm with better PAE can immediately bring down the amount of Pi-fertilizers used in agricultural systems, the development of enhanced internal PUE crops holds a long-term and more desirable sustainable solution [113]. It has been suggested that improving internal PUE (i.e., economic use of P in plants) for the better utilization of already available internal Pi pool for biomass production could prove a more resource efficient approach in plants [114]. For example, high concentrations of P in cereal grains (largely present in the indigestible bound form as phytate) remains underutilized by human and animals. Therefore, developing cereal crop genotypes that accumulate less phytate in their grains but with similar yield potentials would lower P extraction from soils, requiring subsequently less Pi-input to restore soil P balance in the next crop cycle. Although external PUE accessions are known for several crops such as maize, rice, legumes, *Brassica*, more efforts should be made to identify such germplasm for all crops [115]. For identification of high PUE crop accessions, root architectural traits for enhanced Pi foraging capacity, such as increased number of lateral roots/lateral root branching, denser and longer root hairs, more crown/proteoid/cluster/adventitious roots with shallower growth angles of growth have been suggested [24,112]. For example, both longer and more dense root hairs are an attribute of Pi-efficient genotypes of common bean [28]. More lateral roots by plants under Pi deficiency improve possibilities of P scavenging [112]. However, such phenotypes may not always confer increased Pi uptake capacity in all crops. Further, monitoring these traits in the soils remains highly challenging. While increased branching capacity of plant roots may enhance their nutrients foraging capacity, this approach might only partially mitigate the P demand due to the heterogenous distribution of P in the topsoil. Therefore, engineering plants roots responding only to P-enriched patches in the context of a higher number of lateral roots or localized higher root hair density in low P soils may offer a better strategy [41,116],

To identify high PUE genotypes, the rhizosphere can be further analyzed for root exudates, mainly for the protons, organic acids, and hydrolytic enzymes. Pi-efficient genotypes in several plant species may release a higher volume of organic acids in the rhizosphere than the Pi-inefficient genotypes [117]. In a recent study, Wen et al. (2019) investigated root functional traits under Pi deficiency in 16 crop species and reported that substantial inter-specific variations exist for these traits among the species[118]. For examples, species with thinner roots relied more on intense root branching for Pi scavenging whereas species exhibiting thicker roots depended more on the secretion of root exudates for the mobilization of bound P in the rhizosphere [118]. Similarly, Lyu et al. (2016) reported that legumes such as white lupin and chickpea, lacking the fibrous roots (present in cereal species) relies more on exudation of organic acids to mobilize Pi in the rhizosphere than wheat and maize[117]. A root exudation index has been proposed to be useful biomarker for the identification of genotypes with enhanced PAE [45]. However, during such screening, care must be taken as this trait is insufficiently consistent and may vary among plant species. For instance, Pearse et al. (2007) reported that despite the exudation of citrate, pea could not mobilize Pi from Al-P and Fe-P complexes [119]. It has also been observed that organic acids become unstable in both acid and calcareous soils and quickly lost after degradation [120]. Several attempts have already been made to improve PUE in multiple crops. In this context, Zhang et al. (2014a, b) have provided information on candidate genes that have been been explored to improve PUE in different crops [9,121]. Although genotypes with the enhanced secretion of organic acid in the rhizosphere seems an exciting choice, however, it has been reported that in some species the metabolic investment of the plants in producing these exudates is quite substantial [122–124]. In some Proteaceae members and white lupin, the volume of secreted products may amount for ~ 25 % of whole plant dry weight [125][126]. In white lupin, up to 25 % of total photosynthates are diverted as sucrose to cluster roots by Pi-depleted plants to support the enhanced root exudation in the rhizosphere [127]. Especially under Pi stress, rice root exudates attributed to 2-3 % of total plant biomass [128]. For minimizing the carbon costs, the alternative respiratory pathway that is non-phosphorylating in nature (cyanide resistant pathway and rotenone insensitive pathway) should be focused. This pathway allows respiration to proceed without depleting phosphate or adenylate pools and is induced under Pi-deprived conditions [129][7]. Similarly, shifting the biomass allocation towards metabolically efficient root classes, such as adventitious roots, can also enable us to efficiently utilize the soil Pi reserve [130].

Alternatively, improving more efficient internal P usage under its unavailability offer a better solution. Genotypes with effective P mobilization within plants, such as its mobilization from mature/senescing organs to newly emerging/actively growing organs or its recycling between vacuoles and cytoplasm for most crops, must be identified. Akhtar et al. (2008) demonstrated that efficient Pi internal mobilization contributed to the high PUE observed in some *Brassica* cultivars[131]. Early growth and development of germinating seedlings are supported by seed stored P[132]. The high amount of seed/grain P in cereal crops remains under metabolized by humans and monogastric herbivores [132]. Therefore, developing crops with significantly lower seed phytate levels is often advised. However, low phytate levels may lead to compromised seed vigor, and targeted fertilization may overcome such a situation. For example, seed coating with Pi-fertilizers, especially the nano-formulations (such as nano-DAP) could provide a viable solution to compensate low seed phytate levels[133]. Nonetheless, more investigations are needed to create such combinations of modified crops and nano-P formulations to assess their performance in agriculture. Another area to improve internal PUE is by using specific phosphatases involved in internal Pi re-allocation. Acidic phosphatases (APases) are known to libertate Pi from its monoesters in a mildly acidic pH range[52,134]. PAPs constitute the largest family of APases. Several PAPs are known to activate under Pi limitation conditions in plants. Activation of PAPs in shoot marks their importance in mobilization of Pi form intracellular monoesters and anhydrides during Pi deprivation or leaf senescence [134][92]. Similarly, activation of root-specific PAPs help mineralize Pi from its extracellular organic compounds and in-soluble inorganic complexes in the rhizosphere. Although little is known about phosphatases involved in internal re-allocation of Pi, Tang et al. (2013) have reported activation of such a gene, *LaSAP I,* in the roots of Pi-starved white lupin[135]. An *Arabidopsis thaliana* PAP, AtPAP26, has been found to be critical for vacuolar Pi recycling during Pi deprivation and leaf senescence [136][45]. Similarly, transcript abundance analysis of *AtPAP17* gene using Klepikova Atlas, available at TAlR revealed its highly specific high expression in the senescent leaf (https://www. arabidopsis.org/servlets/Tair0bject?id=38 I 09&type=locus). Such a gene could be an ideal candidate for investigating its role in determining internal PUE. Alternatively, enhancing PUE by tweaking plant metabolism to lower P demand, as described in the earlier section, can be explored. In this context, metabolic adjustments done by the members of Proteaceae under Pi deficit conditions are fascinating. One of the critical adjustments undertaken by these members includes an investment of lower Pi resources in rRNA synthesis, especially plastid rRNA. This adjustment does not favor the production of those enzymes which requires P-containing metabolites during carbohydrate metabolism. It also leads to delinking of growth from the synthesis of photosynthetic machinery during early leaf development. Altogether, these adjustment have been found to contribute to a high photosynthetic PUE in the Proteaceae species in comparison to Arabidopsis and crops [137]. Further, the role of sugar signaling in controlling the scale of transcriptional activation of selected PSI genes is exciting and demands further research for a better understanding on the role of sugars in controlling PUE.

7. Harnessing Microorganisms for Enhanced Phosphorus Acquisition in Plants

Soil-based microorganisms are important for mobilizing tightly bound soil P to promote its availability in rhizosphere [116]. Two reactions determine Pi fixation and uptake in the soils. The first process involves fixation of **Pi** onto soil particles, whereas the second involves the solubilization of bound Pi from the available inorganic complexes and organic P present in the soils[138]. Over decades, the frequent use of chemical fertilizers has impacted soil health [139]. Long-term P fertilization adversely affects soil fungal and bacterial diversity in croplands [140,141]. Excessive fertilization also leads to problems like inhibition of substrate-induced respiration by acitidione (activity observed in bacteria) and streptomycin sulfate (activity observed in fungus) [142]. Similarly, triple superphosphate application led to reduced microbial respiration and metabolic quotient [143]. Considering the side-effects of excessive Pi-fertilization, more environmentally benign approaches such as use of Pi-solubilizing microbes (PSMs) as microbial inoculants are routinely carried out to improve crop production **(Fig. 3)**.

A vast number of microbial species have been identified with excellent Pi-solubilizing capacity. Such diversity includes bacteria, fungi, actinomycetes, and some algal species. Among bacteria, *Pseudomonas* and *Bacillus* species are the most well-known Pi-solubilizers. *Rhodococcus, Arthrohacter, Serratia, Chryseohacterium, Gordonia, Phyllohacterium, Delftia, Azotohacter, Vibrio proteolyticus, Xanthohacter agilis, Xanthomonas, Enterohacter, Pantoea,* and *Klehsiella* are some of the other significant Pi-solubilizing bacteria [34,144–146]. Another excellent example of a Pi-solubilizing bacterium is the symbiotic nitrogenous rhizobia *Rhizobium leguminosarum*. Besides nitrogen fixation, this bacteria has been reported to improve plant nutrition by mobilizing Pi from its inorganic and organic forms [147]. For a comprehensive account of the role of PSMs and strategies involved in facilitating Pi uptake [148]. Pi-solubilizing fungi consist of about 0.1 to 0.5 % of the total fungal populations in the soils [149]. Fungi are more suitable because these do not lose their Pi-solubilizing capabilities under laboratory conditions, which often limits the use of bacteria [149][150]. Fungi traveling more distance (even beyond Pi depletion zones) in the soils via their hyphae greatly increases the possibility of P scavenging. AMFs directly deliver scavenged Pi to the root cortical cells [151]. In return, fungus obtains carbohydrates and lipids from the plant [152]. Almost 80 % of plant species are known to have mycorrhizal associations [153]. Hence, the solubilization of P present in the soil can be achieved more efficiently by fungi than bacteria [154]. Since AMFs are naturally present in most soils, it is often difficult to notice their beneficiary role in Pi uptake in field conditions. Nevertheless, laboratory-scale pot experiments involving root colonization by AMF isolates under sterile soil conditions have revealed their positive role in Pi uptake by plants [155,156]. Among the filamentous fungi, *Aspergillus* and *Penicillium* species are the most significant Pi-solubilizers in the soils [157–159]. A few *Rhizoctonia solani* and *Trichoderma* strains have also been reported as efficient Pi-solubilizers [160,161]. Recently, Srivastava et al. (2021) reported the positive role of *Glomus* species inoculation on barley seedlings' growth under the P-optimum nutritional regime[100]. The AMFs-inoculated barley seedlings accumulated higher biomass with less Pi accumulation. Authors observed significantly enhanced PUE in the AMFs-colonized seedlings over their non-AMF controls. While low Pi conditions support plant-AMFs interaction, high soil P conditions are often unfavourable for mycorrhization, although to varying degree in different species[162][163][164][165]. It is important to mention that the degree of success between plant-fungus association is influenced by host genetic factors as well; which tend to vary greatly with the change in the host species. A better understanding of the mechanisms underlying mineral uptake in croplands, especially host factors influencing plant mycorrhiza-mediated mineral nutrition, could be used to develop strategy for a resource-efficient sustainable agricultural system. Two recent review articles published by Sawers et al. (2017) and Kobae (2019) are recommended for further reading to understand the dynamics of the plant-fungus symbiotic associations [152,166]. Altogether, the collected evidence demonstrates that the native microbes in the rhizosphere help improve nutrient uptake. However, their commercial application as bio-inoculants has produced mixed results. One of the reasons for this observation could be that an alien microbe species, used as a bio-inoculant, can compete with native microbes of the rhizosphere and limit their overall nutrient-use efficiency. Therefore, knowing native microbe populations associated with different crops is a prerequisite before using any microbe as a bio-inoculant. Recent metagenomics studies providing insights into the structure and biodiversity of rhizosphere microbiomes for different crops in different soils are encouraging. Such information is useful to understand coevolved diversity and host-microorganisms dynamics. For example, Chalasani et al. (2021) recently showed that non-symbiotic *Rhizobium* species predominantly colonizes pigeon pea roots, rather than symbiotic *Bradyrhizobium* species in Indian soils [167]. Additionally, the data provided insights into the factors controlling pigeon pea bacterial community structure. Plant fraction, followed by developmental stage, soil type, and although the least important yet still a significant factor, plant genotypes influenced the bacterial community. Similarly, a genome-wide association study targeting to understand population-level microbiome analysis of the rhizospheres of 200 sorghum genotypes revealed a putative plant locus that control the heritability of the rhizosphere microbiome [168]. Such data would be useful in devising effective ways to utilize species-specific bacterial/fungal partners to improve overall fertilizers' nutrient-use efficiency.

8. Nanofertilizers: Advancing Precision Nutrient Delivery for Sustainable Agriculture

The currently used chemical fertilizers have a major problem with their agronomic efficiency [169]. Currently used mineral fertilizers are inefficient and needed in substantial quantity to support agricultural systems. Moreover, the granular forms need a massive amount of water for their dispersal. Advanced technology-based solutions such as nanotechnology are being explored to improve the delivery of the plants' macro and micronutrients (such as P, N, and Zn). For instance, the application of urea-hydroxyapatite nanohybrids (for the slow release of nitrogen) in the rice fields significantly enhanced the agronomic use efficiency of urea. The field trial data clearly showed that the nanohybrids were translated directly to enhanced plant availability and growth while reducing the nitrogen content used by up to 50 % [170]. Similarly, we also noticed the promotion of vegetative growth of monocot and dicot species after applying a reduced amount of cryo-milled diammonium phosphate nanoparticles (nDAP) over granular OAP [133]. In this novel approach, the cryo-milling method was used to produce ~ 5000 times smaller particles of nDAP over the granular OAP (cDAP). A comparative study using different concentrations of nDAP and cDAP to investigate their effects on plant growth showed cDAP grown tomato and wheat seedlings being outperformed in biomass production by their equimolar nDAP grown counterparts. The nDAP grown seedlings consistently accumulated more Pi than their cDAP grown controls in all concentrations, indicating the beneficial role of reduced-sized Pi-fertilizers in plant growth promotion [171].

9. Unveiling the hidden marvels: An In-Depth introduction to mycorrhizae

With the advent of fertilizers and pesticides, one of the common strategies employed by mankind is to apply them in larger quantities which will untimely lead to the depletion of the nutrient reserves by the end of the next century. This necessitates the shift to management practices that are conducive to the environment. Various research studies suggest the potential use of bio-fertilizers including microorganisms like Arbuscular Mycorrhizal Fungi (AMF), which can form a better alternative to curb this problem and enhance crop productivity. AMF originally belonging to the phylum Mucoromycota, and subphylum Glomeromycotina, are believed to be the planet's most prolific plant-fungal relationship [172]. Arbuscular Mycorrhizal Fungi are ancient, presumably asexual, widespread, mutualistic endosymbiotic fungi that have successfully formed a friendly association with almost 80 % of the land plants including many of the bryophytes, some pteridophytes, few gymnosperms and most of the angiosperms [173]. The lack of any sexual reproduction mechanism renders the phylum a phylogenetic mystery. The documentation of arbuscules in *Aglaophyton majus*, vesicles *in Rhynia Gwynne-Vaughan* and recent evidence of arbuscules and vesicles *in Horneophyton ligneria* suggest that the fungi are known to have a co-evolution history that dates back 400 million years ago in the time frame of the Early Devonian period [174–177]. This hints toward the potential role of AMF in the invasion of the terrestrial land by the aquatic ancestors. Even though evidence for mutualistic

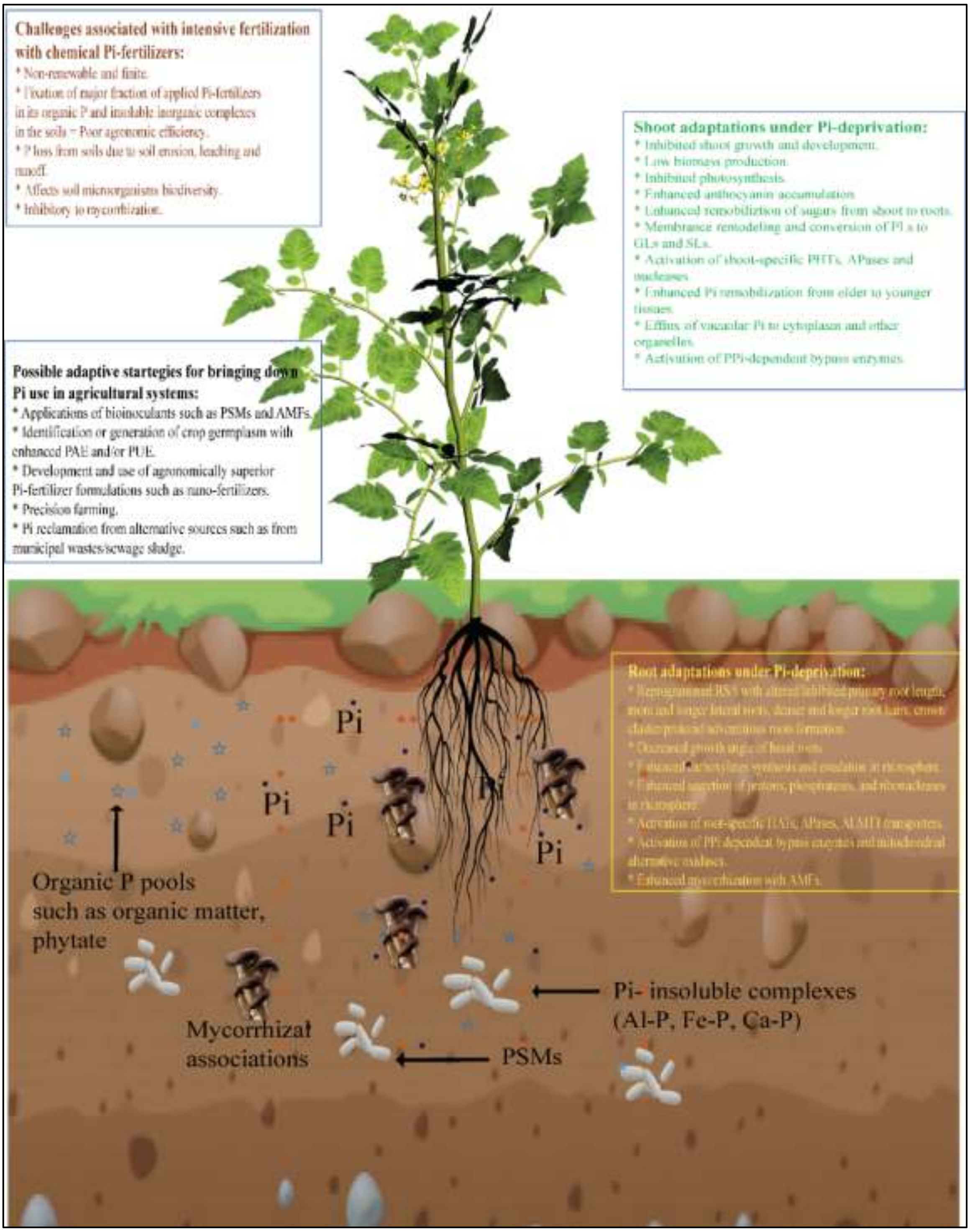


Fig 3: *Schematic representation of phosphate starvation responses and strategies adapted by plants under low Pi to circumvent phosphate-deprived response. The figure talks about salient features related to phosphate uptake (PHTs and HATs), homeostasis, and mobilization scenarios in plants along with the alternate Pi-uptake/recycling approaches. Under Pi deprived condition, morphological changes occur in roots at RSA (root system architecture) level. While in shoots, modulations in terms of length and biomass are observed. Although AMFs (arbuscular mycorrhizal fungi) and PSMs (phosphate solubilizing microbes) can help in the mineralization of organic P, unique approaches related to Pi uptake can pave the way for efficient P usage and for a sustainable agrarian system.*

association remains largely obscure, the identification of arbuscules and vesicles in these fossils is congruous with the symbiotic association that regulates the growth and development of plants. Because the fungus lives inside plant root cells, this relationship is both biotrophic and mutualistic in nature. Nonetheless, exceptions such as ecto-, ericoid, and orchid mycorrhiza along with non-mycorrhizal plant species also exist in nature but are primarily associated with extreme soil conditions [178]. It is noteworthy to mention that due to the difficulty in culturing AMFs, it is anticipated that a large diversity of AM species is yet to be identified and explored.

10. Arbuscular Mycorrhizal Fungi (AMF): Facilitators of Nutrient Exchange

The AMF enable the plants to intrude into the nutrient depletion zone and assimilate scarce nutrients like phosphate(P), Nitrogen(N), and Zinc (Zn) from unabsorbable forms [179]. In order to compensate for this, the fungi acquire photosynthetically fixed carbon and lipids [180] from the plant partner that help the fungus for its survival and propagation. AMF colonization is widely thought to enhance biomass accumulation which is attributed to the enhanced nutrient uptake and increased photosynthate production. AMF not only contribute to the uptake of soil nutrients but also confer improved resilience to abiotic stresses. It also aids to mitigate the environmental constraints that hinder plant growth and development by triggering the AMF-induced tolerance mechanisms. It was also shown that AMF can attenuate the free radical damage caused by abiotic factors by boosting the host plant's defence mechanism [181,182]. To deal with the drought stress and reduce reactive oxygen species (ROS) burst, AMF operates two separate pathways- AMF responses and host responses pathways. There occurs a short oxidative burst in the mycorrhizae-containing cells during early symbiosis and degradation [183]. In the AMF responses pathway, AMF fungal extraradical hyphae, AMF antioxidant genes and fungal aquaporins (AQPs) contribute to the water absorption and translocation to the host hence, alleviating the drought stress. In the latter pathway, the antioxidant protection mechanisms are increased, particularly, the polyamine and fatty acid homeostasis. Li et al. (2013) earlier reported the localised expression of the aquaporin gene near the region of hyphal penetration [184]. The strategy of increasing certain enzyme production, accumulation of proline, increased nutrient uptake and reduced uptake of sodium and calcium helps to persist during the salinity stress during symbiotic interaction [185–187]. During elevated levels of toxic metals, the glomalin produced by the AMF is thought to slow down its transport [188]. An insight into the role of AMF to resist pathogens provide plants resistance to biotic stress. The intimate relationship between the fungal and plant partner flourishes once the nutrient availability is less. The highly branched hyphal network that extends beyond the nutrient depletion zone facilitates the nutrient transfer to the plant partner. The genetic underpinnings of functional compatibility propound that the plant-fungal genotype combination largely determines how much the plant profits from the association.

11. Unraveling the Tale of the Plant-AMF Symbiotic Alliance

To establish a mutually beneficial plant-AM relationship, both plant and fungal partners endure significant morphological and physiological changes. AM fungi produce a vast hyphal network called extraradical mycelium (ERM) that surveys the soil, gathers nutrients, and then delivers them to the plant root via the intraradical mycelium (IRM)[189]. In contrast to IRM, ERM has relatively thinner roots, which enable plants to acquire the nutrients from the soil where the fine roots can’t reach and hence contributes to the main fungal biomass. Whereas, the IRM forms the main fungal body that extends throughout the root cortical cells. Root colonization is tightly regulated both physiologically and developmentally. It occurs through different phases including the asymbiotic, presymbiotic, infection and symbiotic phases. In the asymbiotic phase, fungal spores germinate and have limited hyphal development without a host plant. The presymbiotic stage represents germination of fungal spore, hyphal branching and cell proliferation. The growing hypha then intensely branches and ramifies close to roots in response to root exudates, including strigolactone [190] and differentiates to form appressorium at the root surface. During the infection phase, infection hyphae arise from appressoria and penetrate the root epidermis. This initial hyphal entry into roots is followed by extensive intraradical hyphal development during the symbiotic phase which culminates in the arbuscules formation in the inner cortical cells. It is now known that the formation of a narrow structure, designated as the prepenetration apparatus (PPA), in a root epidermal cell precedes the fungal entry into the roots, indicating the host’s preparedness to undergo into the relationship [191]. IRM generally forms specialized tree-like heavily branched terminal structures, called arbuscules, or hyphal coils [192]. In response to arbuscules formation, the host also modifies its plasma membrane to cover the fungal structure. Such membrane is called perifungal membrane or periarbuscular membrane (PAM). Arbuscules never enter the host cell’s cytoplasm and plant-fungal membranes demarcate the boundary, an apoplastic compartment in the root cortex. This structure represents the symbiotic interface in the root cells and mainly facilitates the metabolic exchange between the two organisms. Due to the periarbuscular membrane that sheaths the fungi, they are not entirely assimilated by the plant organelle [193]. The peri arbuscular membrane encompasses the phosphate transporter genes and is simultaneously expressed along with the arbuscular formation. The arbuscular generation on the other hand requires the concerted differentiation and rearrangements of both the symbionts. Roth et al. (2019) identified membrane tubules and PAM to be involved in the arbuscular rearrangements[194]. They also proposed the role of extracellular vesicles in the arbuscular degeneration. The observation of high phosphate levels in the cells along with the broad interface suggests localised phosphate transport [195]. Upon reaching the surface of the root, the hyphae differentiate into hyphopodium, which causes interesting subcellular rearrangements of the underlying cells. The cytoplasmic bridge thus formed across the vacuole which is lined by membrane cytoskeleton and ER, direct the fungus through the cell.

12. Hormonal Changes Triggered by Arbuscular Mycorrhizal Fungi (AMF) Colonization

The establishment of AM symbiosis is closely intertwined with plant hormone homeostasis. Various studies demonstrated that nearly all phytohormones including have a dominant role in different stages of symbiotic signalling pathways. The pre-symbiotic signalling is made effective with the release of strigolactone that attracts the AMF towards the plant roots. Many in-vitro studies show that strigolactones enhance spore germination and subsequent mycorrhizal development, especially under low P concentrations [196][190]. The diffusible fungal factors essential for Ca^{+2} oscillations are supplied by the strigolactones to the rhizosphere prior to colonization [197][198]. It can also enhance fungal metabolism thereby generating ATP for fungal growth and branching. The regulatory effects of strigolactones depend largely on its concentration [199]. Choi et al. (2020) found that SMAX1 suppress symbiosis and established interlink between the strigolactone and karrikin signalling pathways [200]. Abscisic acid is another apocarotenoid hormone that promotes root colonization and arbuscule formation. The ABA pre-treatment is proven to enhance the spore viability and hyphal branching. While it is also proven to act as inhibitor in plants with fair colonization [201,202]. The mycorrhizal colonization under ABA administration requires a PP2AB'1 (holoenzyme subunit), whose mutation reduces the AM colonisation by 50 % with no discernible impact on the formation of fungal structures [202]. The gaseous plant hormone, ethylene is thought to act antagonistic to ABA, but several studies show various discrepancies and hence its effects largely remain elusive [203,204]. While inhibitory effects of GA on arbuscular development is remain obscure [205–207]. The gibberellin-DELLA complex also has a significant effect on mycorrhization with DELLA proteins showing an inhibitory effect on GA responses. In crosstalk with ABA and JA via orchestrating the DELLA proteins it also enhances arbuscule generation, whereas. Auxin is believed to positively regulate the strigolactone biosynthesis genes and hence promote mycorrhizal development [208]. The enhanced auxin levels during early colonization stages indicate its positive role in mycorrhization. Even though it was believed that auxin and cytokinin act antagonistically, recent evidence by Cosme et al. (2016) indicates cytokinin from both shoot and root enhances mycorrhizal symbiosis [209]. The effects of Jasmonic acid remain predominantly unstudied with very few in-vitro studies reported. He further reported other signalling compounds like coumarin aid mycorrhization during the early stages. The mycorrhizal colonization in the host plant elicits 'priming', a mechanism that helps the plant from both biotic and abiotic stresses [210][211].

13. Mediators Promoting the Association with Arbuscular Mycorrhizal Fungi (AMF)

Plants under low nutrient conditions secrete certain plant hormones like strigolactone and flavonoids, often called 'branching factors' from the plant root. The signalling molecules from plants also result in the exudation of certain short-chain chitin oligosaccharides (COs) and lipochitooligosaccharides (LCOs) that induces symbiotic and immunity responses. These signalling molecules produced by the fungus aid them to get accommodated in the host plant by inducing calcium spiking. It also stimulates lateral root growth, and branching and induces the expression of symbiotic (SYM) genes in the host through the common symbiotic signalling pathway (CSSP). It was long been assumed that the COs and LCOs exhibit diverse behaviours in eliciting symbiotic responses. But, Feng et al. (2019) in *Medicago trunculata* concluded that both COs and LCOs are essential for symbiotic establishment, whereas COs independently suppress immunity responses[212]. It is also found that receptors such as Lysine motif receptor like kinases (LysM-RLKs) are necessary for the interaction with signaling molecules such as strigolactone, CO and LCO's. CCaMK which are responsive to calcium, phosphorylates and induces the transcription factor CYCLOPS, which results in the activation of transcriptional regulators, like GRAS transcription factor RAM1. Calcium calmodulin-dependent kinases (CCaMK) along with the action of a putative cationic channel in the nuclear membrane and leucine-rich repeat (LRR) receptor-like kinase induce Ca^{2+} spiking. Recent studies in *Medicago trunculata* suggest that DMI1/2-dependent cytoplasmic spiking can be observed in the root hairs proximal to the branching fungal hyphae. Chabaud et al. (2010) proved that the Ca^{+2} oscillations are triggered by diffusible fungal factors and host cell-cell communications[213]. There occur other pathways that are different SYM pathways which suggest that the AM fungi elicit a wide array of active molecules that can trigger different cellular targets [214][215].

14. Insight into the mechanistic aspect of AMF colonization in plants

The initiation or deciphering of calcium-spiking includes a sub-set of genes including SYMRK (symbiosis receptor-like kinase) which is essential for the perception of the signalling molecules and functional symbiosis. SYMRK acts as a co-receptor in the perception of COs and LCOs certain secondary messengers which result in high-frequency calcium spiking around the nucleus. For successful nodulation, the cross-talk of many genes is quintessential among which SYMRK works like a signal transducer. SYMRK is a protein kinase localised in cell-membrane which possesses an extra-cellular malectin-like domain (MLD), leucine-rich repeat (LRR) domains towards extracellular region, transmembrane and a Ser/Thr kinase domain in cytoplasmic side. It helps in the early events of arbuscular mycorrhization and symbiosome formation. The Myc and the Nod factors which are N-acetylglucosamines also get recognised by the MLD. This promotes microbe interaction and cell-cell communication. Even though the molecular mechanism of SYMRK is unclear, it plays a major role in recruiting previous molecular mechanisms to regulate symbiosis. The mutation in SYMRK affects both nodulation and effective colonization which highlights the role of the gene in the fungal signalling cascade. The overexpression studies by Zhijing et al. (2012) imply that SYMRK promotes colonization and biomass in tobacco root[216]. The orthologues of the gene have been identified in legumes, but its role in non-leguminous plants such as in tomato is yet to be explored.

15. Regulation of mycorrhizal development and root colonization

Mycorrhiza formation requires a well-coordinated communication between plant and AM fungal partner. While both morphological and physiological changes associated with both host and fungus have been well-demarcated, the molecular complexity of processes involved in AM symbiosis is less elucidated. Although it is important to note that several host and myc factors that help coordinate plant fungus interaction have been identified over the past few decades. A concerted effort by many studies involving transcripts profiling and in situ hybridizations of RNA has revealed the identity of several novel genes controlling hyphal growth and development involved in arbuscule function and development.

16. Role of AMF in the light of P nutrition

The extent of mycorrhizal colonisation is inseparable from the amount and form of P in the soil. Even though it is a macronutrient required in relatively larger quantities, Pi deficiency is observed in most of the soil around the globe and levels of available Pi seldom exceed 10 μM even in fertile soils [53]. Moreover, the availability of Pi in soil (~2μM) is severely limited when compared to that in plant tissues (5–20mM). The availability of Pi is a concern as most of it is fixed in the soil or is in constant interaction with other inorganic and organic surfaces in the soil [217]. The inseparable mineral-Pi complexes in the soil also render the plants difficult to assimilate [69]. After the successful mycorrhizal colonization, plants unveil two Pi-uptake strategies at the soil-root interface, DPU (direct phosphate uptake) or MPU (mycorrhizal Pi uptake pathway). In the DPU pathway, Pi uptake is mainly through the epidermis and root hairs under Pi stress. Whereas in the MPU pathway, the exchange of Pi occurs between the interjection between the fungal mycelium and cortical cells. The transfer of Pi mainly occurs with the help of DPU but once the colonization is established by AMF in the roots, then MPU pathway operates. DPU and MPU pathways can be anticipated to be carefully adjusted by interacting signal transduction pathways in a changing root environment. Since changing Pi concentration in plant cells can cause adverse effects in plants, maintaining adequate Pi homeostasis is crucial for plant growth and development. AM-mediated Pi acquisition from the rhizosphere thus operates where Pi is taken up against the electrochemical gradient into the ERM with the help of ABC transporters and H^+-ATPases which rather spans the membrane. Pi is acquired by the MPU through H^+/Pi or Na^+/Pi symporters present in the AMF extraradical hyphae, in contrast to the DPU where Pi is transported by $H^{+/}$Pi symporters on the epidermis [218][219]. The Polyphosphate (polyP) which builds up in the hyphae modulates the cytoplasmic Pi levels and gets transported along the hyphae. The occurrence of the positive mycorrhizal P response, high mycorrhizal growth response (MGR) and higher total P in the AM inoculated plants hints at the existence of the MPU. The downregulation of the phosphate transporter genes involved in the DPU and the upregulation of the MPU-specific transporter genes during mycorrhization propounds that both these pathways are not additive although the mechanism behind this is yet to be explored [220–222]

17. The journey of Pi through the Phosphate transporters

The supply of high phosphate is detrimental to plants and it regulate the pre-symbiotic signalling and hyphopodia formation [215][196]. High levels of phosphate are proved to inhibit the calcium oscillations and symbiotic pathway [223]. The periarbuscular membrane encompasses the phosphate transporter genes and is simultaneously expressed along with the arbuscular formation. The arbuscular generation requires the concerted differentiation of both the symbionts. The observation of high phosphate levels in the cells along with the broad interface suggests localised phosphate transport in these arbuscules [195]. The mutant studies in Medicago and Rice shows that abhorrent P-specific transporters *mtpt4* and *OsPT11* are involved in arbuscule degeneration [224][225]. The plant Pi transporters, grouped mainly according to subcellular localization and sequence variability and falls into five subfamily groups: PHT1, PHT2, PHT3, and PHT4 out of which the Phosphate Transporter1(PHT1) subfamily is considered important. The premature arbuscule degeneration (PAD) observed in the studies within Medicago (*pt4*) and rice (*pt11*) mutants indicate that these PTs are quintessential in the sustenance of the symbiosis, phosphate uptake and mainly in arbuscular maintenance. Xie et al. (2022) further discovered that PT7 containing an SPX domain in *Rhizophagus irregularis* generally promotes the arbuscular symbiosis and phosphate exchange[226]. The discovery of MYB1 transcriptional regulator by Floss et al. (2017) in Medicago roots suggests its role in arbuscular suicide[227]. The *myb1-pt4* double mutant arrested the arbuscular degradation while overexpression of MYB1 hydrolyses the proteins like chitinase, lipase and protease that enact the degradation. In order for them to enact, the NSP1 (a GRAS transcription factor gene) and DELLA (GRAS protein) should interact with the MYB1 [228]. It is noteworthy to mention that the same transcriptional regulator that causes arbuscular degradation can also facilitate its formation. On the contrary, DELLA is also thought to intermingle with the prime transcription factor CYCLOPS which promotes arbuscular branching. A crucial component that prevented the underperformance of the symbiotic association for millions of years can be related to the high arbuscular turnover. Transcriptomic profiles that are distinct to different tissues and stages of development are controlled by specific cis-acting elements. In eudicots, MYCS and P1BS are regarded as unique motifs required for the constitutive activity of Pi transporters.

18. The master control of Pi transport

The plants combine Pi status with fungal colonisation and arbuscular growth to maintain a favourable connection throughout symbiosis. Plants have evolved a group of Phosphate Inducible genes (PSI) to mitigate the Pi deficiency and to act as a gateway for the available Pi in the soil. It’s interesting to note that there occurs a ubiquitous P steering vehicle called (Phosphate Starvation Response) which falls as a member of R2R3 MYB transcription of the PSI genes. The central regulatory mechanism of PHR (Phosphate Starvation Response) factors contain important regulators with SPX domains in order to locally track the quantity of phosphate exchanged and to maintain the Pi homeostasis during AM symbiosis. These phosphate sensors work by modulating the PHR at altered phosphate conditions by selectively interacting with the inositol polyphosphates (PP-InsPs) that later interact with the SPX domains. The SPX members are known to interact with the PHRs only under Pi-deprived conditions by the activation of PSI genes. This reduces the arbuscular symbiosis and expression of AM-related genes. Whereas, under Pi adequate conditions, SPX undergo proteasomal degradation rendering PHR to freely bind with the promoter of PSI genes [229]. Das et al. (2022) further explored the multidirectional control circuit between the SPX and PHR and established that additionally many AM-induced transcription factors are expected to stabilize PHR2 interaction [230]. Das and the team also uncovered unknown PHR2 sites implicated in the initial phase of symbiosis[230]. Their findings highlight that PHR is rather conserved in legume Lotus japonicus and predicted that the strigolactone biosynthesis might not be controlled by PHR. The ChIP-Seq of PHR targets in Lotus japonicus by Das et al. (2022) further confirmed that PHR2 is essential for mycorrhization and nutrient exchange by activating transporters including PT11. Liao et al. (2022) further extended the studies to tomatoes where SPX1 suppressed the arbuscular formation and Pi uptake by complexing with PHR [231]. The authors also revealed that the SlSPX1 silencing alone renders the Pi uptake ineffective, indicating a dominant role of it in symbiosis. Wang et al. (2021) proved that MtSPX1 and MtSPX3 as low Pi-induced regulatory genes during symbiosis which have roles in enhanced root colonization by strigolactone production [232]. Thus, it can be concluded that the role of SPX with respect to mycorrhizal colonization largely remains obscure and also varies in different plant species.

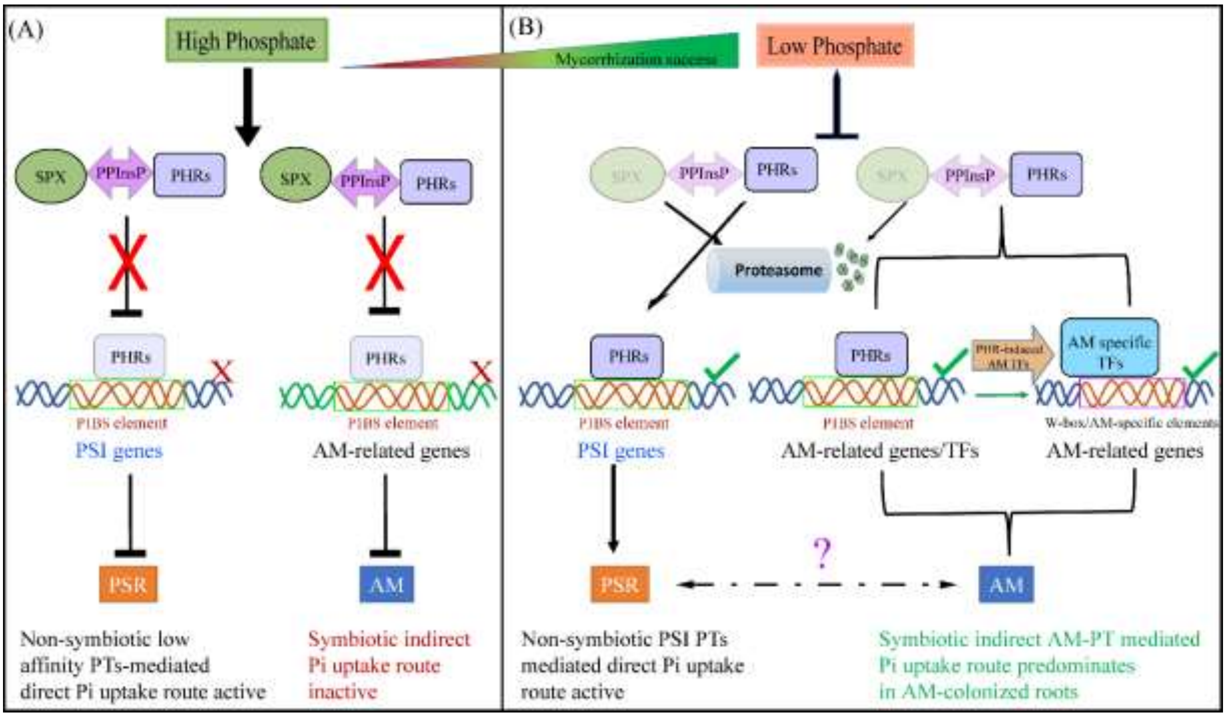


Fig 4: *SPX-PHR centered network orchestrates arbuscular mycorrhizal symbiosis under differential phosphate regime.*

In AM-colonized roots, two pathways operate for Pi uptake; the direct Pi-sensing pathway involving host root machinery and the indirect and predominant route involving fungal hyphae. The indirect route transfer nutrients directly to host root cortical cells. Several host regulators, such as WRINKLED5a/b, REQUIRED FOR ARBUSCULAR MYCORRHIZATION 1 (RAM1) , REQUIRED FOR ARBUSCULE DEVELOPMENT 1 (RAD1) [180,233,234], and MYB1 [227] are known to regulate different aspects of AM symbiosis, including pre-contact signaling, root colonization, and AM function. While GRAS family transcription factors (TFs) such as RAD1 and RAM1 regulate hyphal branching, WRINKLED5a (WRI5a) is known to control lipid biosynthesis and transfer during mycorrhizal symbiosis [235]. Similar to AM symbiosis, Pi homeostasis is under the tight control of the antagonistic activities of host SPX [named after SYG1 (suppressor of yeast gpa1), Pho81 (CDK inhibitor in yeast PHO pathway), and XPR1 (xenotropic and polytropic retrovirus receptor)] proteins[229] and phosphate starvation response regulator (PHR) TFs in plants. Under low Pi conditions, PHRs activate phosphate starvation response (PSR) by directly binding to PHR1 binding sequence (P1BS) elements in the promoters of phosphate starvation inducible (PSI) genes, including phosphate transporters **(Fig. 4)** [236]. Under high Pi conditions, SPX proteins physically interact with PHRs and negatively regulate their activity **(Fig. 4A)**. Whereas low Pi conditions support both PSR and AM-symbiosis, the mechanism of how host PSR regulators influence the two Pi-uptake pathways and how increasing soil Pi levels impair mycorrhizal colonization remained poorly understood.

A new study by Shi et al. (2021) highlights the central role of PHRs in determining mycorrhizal infection success in rice [237]. In this study, a comprehensive yeast one-hybrid-assay screen (YIH) involving 1570 TFs and promoters of 51 AM-related genes revealed a highly interconnected network centered on 266 TFs and 47 target promoters. The authors reported 511 significant interactions involving both AM- and PSR-specific TFs such as WRI5a/b, RAM1, and PHR1/2/3. In line with previous findings, severely diminished fungal colonization in OsRAM1 and OsWRI5a/b mutants confirmed their positive influence on mycorrhization [238][239]. Interestingly, 25 promoters were found to be activated by OsPHRs in YIH. The enrichment of P1BS element in the promoters of most AM-related genes, including OsRAM1, OsRAD1, OsWRI5a, OsAMT3;1 (ammonium transporter3;1) and OsPT11 (phosphate transporter11), delivered the first line of evidence for PHR-regulated mycorrhization in plants. The enrichment of the P1BS element in most AM-related genes promoters, including the well-known AM-specific regulators such as RAD1, RAM1, and WRI5a/b, further supported a positive association between OsPHRs and AM. Experimental validation of OsPHR2 binding to the P1BS element of OsRAM1, OsWRI5a, OsPT11, and OsAMT3;1 promoters further consolidated this notion [229]. The inhibited expression of OsPT11:GUS promoter-reporter constructs upon

P1BS deletion evidenced a direct regulatory role of OsPHR2 in OsPT11-mediated indirect Pi transport in the arbuscule-containing root cells. The authors also noticed a restricted OsPHR2 expression in arbuscule-containing cells, contrary to its ubiquitous expression in the non-colonized Pi-deficient roots. Additional evidence such as the impaired mycorrhizal colonization in Osphr2 single and Osphr1/2-1/3 triple mutant roots and the restoration of this phenotype by expressing wild-type OsPHR2 wild allele in the mutant backgrounds reinforced the idea of PHRs regulating AM symbiosis. This observation was further supported by the reduced transcript levels of OsRAM1, OsWRI5a, OsPT11, and OsAMT3;1 in the loss-of-function Osphr2-1 mutants. On the contrary, AM genes (such as OsWRI5a, OsAMT3 and OsPT11) were found to be induced in the non-AMF colonized OsPHR2-overexpressing roots. A comparatively higher degree of fungal colonization in these transgenic plants than their wild-type controls in Pi-sufficient conditions finally confirmed a central role of PHRs on steering plant-fungus symbiotic association. Consistent with the negative regulatory role of SPX on PHR activity, the authors noticed a significantly lower frequency of arbuscule formation and mycorrhization in the roots of OsSPX1 and OsSPX2 overexpressing transgenic plants [229]. Moreover, the enhanced fungal colonization and arbuscules formation in the Osspx1/2/3/5 quadruple mutant roots, a phenotype reminiscent of OsPHR2 overexpressing lines, further supported the negative influence of SPX proteins on fungal colonization. Together with the previous findings, the activation of an AM-specific phosphate transporter, OsPT11, and de-repression of many PSI genes in either Osspx mutants or OsPHR2 overexpressing plants, even under Pi-sufficient conditions, this study expanded the regulatory role of SPX-PHR controlled PSR machinery beyond Pi homeostasis to AM symbiosis.

The findings of Shi et al. (2021) found excellent support in another study by Wang et al. (2021), which reports the regulatory role of AM-inducible SPX members in controlling fungal colonization and arbuscules degradation in Medicago roots[232,240]. The authors first identified MtSPX1 and MtSPX3 as the low Pi favored AM-responsive genes. Besides P1BS elements, the promoters of both genes also harbor multiple AW-boxes and CTTC elements, the known WRI5a, and RAM1 binding motifs. The de-repression of MtSPX1 and MtSPX3 in WRI5a overexpressing roots, even under high Pi conditions, suggested that WRI5a probably favours mycorrhization by binding to the AW-boxes in their promoters. Further examination of the spatial expression of MtSPX1 and MtSPX3 through their promoter: GUS reporter constructs revealed a highly restricted GUS signal to only arbuscule containing cells, a phenotype reminiscent of OsPHR2 promoter: GUS reporter constructs reported by Shi et al. (2021) [240].

Consistent with the earlier findings, MtSPX1 and MtSPX3 also controlled Pi homeostasis by negatively regulating the activity of MtPHR2 under high Pi conditions [229,240]. Significantly lower root mycorrhization and inhibited arbuscules abundance in the Medicago spx1/3 single and double mutant plants indicated conserved roles of SPX proteins in AM colonization in plants. The authors further quantified the different-sized arbuscules and reported significantly fewer degrading and more large-sized arbuscules in the spx1/spx3 double mutant than in the wild-type roots. The reduced transcription of CYSTEINE PROTEASE3 and CHITINASE, the target hydrolase genes of MYB1, a TF regulating arbuscules degradation, in only spx1/spx3 double mutant plants, and no significant change in the ratio of mature/degrading arbuscules between single spx1 or spx3 mutants and wild type suggested redundant roles of these genes in arbuscules degradation [227]. Because of the inhibited fungal colonization and arbuscules abundance in MtSPX1 or MtSPX3 overexpressing, driven under AM-responsive MtPT4 promoter, Medicago transgenic roots, Wang et al. (2021) decisively confirmed the regulatory role of SPX-PHR in AM symbiosis in plants[232].

The recent studies conclusively demonstrate that the expression of both SPX and PHR proteins shifts from the whole root to arbuscules containing cells upon low Pi-induced mycorrhizal colonization. Under Pi-sufficient conditions, PP-InsP activates SPX members to interact with PHRs for inhibiting their activity **(Fig. 4A).** Under Pi-deficiency, SPX members are degraded through the proteasome pathway, releasing PHRs free for binding to the P1BS of target promoters. Thus, besides activating PSI genes, PHRs also directly activate AM-related genes, through P1BS, under low Pi conditions. Alternatively, PHRs can activate AM-specific downstream TFs, such as WRI5a/b or RAM1, which then initiate the transcription of downstream symbiosis-associated targets, plausibly by binding to AW-boxes and CTTC elements in their promoters, to regulate mycorrhization **(Fig. 4B)**. Thus, SPX-PHR proteins, besides regulating PSR under nonsymbiotic conditions, also regulate AM symbiosis in Pi-deficient plants. However, further research is required to explain how SPX-PHR expression is restricted to only arbuscules containing cells upon AM-colonization. More investigations to understand the mechanism of SPX-PHR mediated plausible integration of direct and indirect Pi uptake routes are also needed.

Material and Methods

Seeds of tomato cultivar (*Solanum lycopersicum* cv Pusa Ruby) was in the most of the experiments. Seedlings were grown under controlled condition in culture room at a temperature of 24 ± 3 °C with a photoperiod of 16 h and 8 h of light/dark period. Intensity of light was maintained at 200 µmol m^{-2} s^{-1}, and relative humidity was kept around 60 %. For Pi starvation experiments, tomato seeds were at first treated with sodium hypochlorite, thoroughly washed and then placed in dark for germination at temperature 24 ± 3 °C. The germinated seeds of radicle size ~1-cm in were transferred to either hydroponic set up in pots containing ½ X Hoagland medium or to Phyta Jars (500 ml capacity, Himedia, India) containing ½ X MS (Murashige Skoog, SRL) solid media containing high phosphate (1.25 mM) and low phosphate (5 µM) concentrations. The main source of phosphate is KH_2PO_4. Equal concentration of KCl was used to compensate for potassium in low phosphate conditions. Seedlings were harvested 15 days post starvation. Samples were rapidly frozen using liquid nitrogen. The samples were stored at -80 °C for further analysis. Each experiment was performed a mini-mum of two times, with at least 15 seedlings per treatment.

2. Screening of tomato germplasm

After germination uniformly sized radicles were transferred to ½ X Hoagland medium containing high phosphate concentration for a period of 11 days under controlled conditions. After that, plants were subjected to Pi starvation by shifting them to low phosphate medium for 15 days. The, samples were collected for end point measurements, morphometric and molecular analyses.

3. Growth condition in greenhouse

Germinated seedlings are transferred to pots containing mixture of moistened cocopeat and soil rite. Plants were first grown for 15-20 days in a culture room under the earlier described conditions. Plants were then transferred to greenhouse and kept for three days to acclimatize under the controlled environment. The acclimatized seedlings were then transferred to soil-filled pots. Image of seedlings were captured using canon high-definition camera (Power shot SX520 HS). For studying the root and shoot length, picture of all the seedlings were taken by HP LaserJet 1536dnf MFP printer with scale and measured by using ImageJ tool. For, counting the number of root hairs, light microscope (Leica M105FC, Germany) was used. For leaf morphology study, scanning was done as mentioned earlier and the data was analyzed using ImageJ tool.

Table 1

Murashige and Skoog media components, pH 5.8	
Macronutrients	**½X MS media, pH- (5.6-5.8), Conc. mg/L**
$CaCl_2.2H_2O$	166.1
KH_2PO_4	170.1
KCl	70
$MgSO_4.7H_2O$	90.345
NH_4NO_3	825
Micro-nutrients	
$CoCl_2.6H_2O$	3.125
$CuSO_4.5H_2O$	0.012
FeSO4.7H2O	13.9
H_3BO_3	3.1
MnSO4.H_2O	8.45
Na_2-EDTA	18.65
$Na_2MoO_4.2H_2O$	18.65
$ZnSO_4.7H_2O$	4.3

Table 2.

Hoagland's complete nutrient solution pH 5.8		
Macronutrients components	**Volume required in ml for preparation of ½x L, High Phosphate (HP)**	**Volume required in ml for preparation of ½x L Low Phosphate (LP)**
KH_2PO_4	1.25 mL (1.25 mM)	5 µL (5 µM)
Ca $(NO_3)2$-$4H_2O$	2 mL (2 mM)	2 mL (4 mM)
1M $MgSO_4$-$7H_2O$	1 mL (1 mM)	1 mL (1 mM)
KNO_3	3 mL (6 mM)	3 mL (6 mM)
Micronutrients components	**Conc. (g/L) for preparation of stock solution**	**Micronutrient (1000x)**
$CuSO_4.5H_2O$	0.08	All the components are mixed together and used 0.5 mL of this stock for ½ x/L working Hoagland solution
H_3BO_3	2.86	
$MnSO_4.H_2O$	1.81	
$Na_2MoO_4.2H_2O$	0.12	
$ZnSO_4.7H_2O$	0.22	
Stock solution (Fe-EDTA)	**Stock solution (Conc. g/L)**	
Na_2-EDTA	Dissolve 26.1g in water containing 19g of KOH	The pH rises to about 7.1. The solution appears red with little precipitation at the bottom. 0.5 mL used from stock for preparation of ½x/L Hoagland solution.
$FeSO_4.7H_2O$	Dissolve 24.9g in water and slowly add this solution with stirring in KOH-Na_2EDTA solution.	

4. Estimation of soluble Pi and P content

Quantification of total soluble Pi was done with plant tissues in root and shoot by using colorimetric based assay involving phosphomolybdate, as illustrated by Ames (1966) [241]. Plant tissue, such as root and shoot was homogenised using liquid nitrogen in mortar and pestle. 30 – 40 mg grounded tissue was weighed and immediately transferred in 1.5 ml microcentrifuge tube (MCT), containing 250 µl of 1 % glacial acetic acid. It was mixed by vortexing and then subjected to liquid nitrogen for approximately 30 sec. The tubes were thawed at room temperature. After that, tubes were centrifuged at 14000 g for 2 min. It was followed by gentle transfer of 50 µl of supernatant i.e., the upper phase to a new MCT. The other solutions (600 µl of 0.42 % acidic ammonium molybdate +

100 µl of 10 % ascorbic acid) were added to the supernatant containing tubes. The mixture was vortexed and incubated at 45 °C for 20 min. Subsequently, 200 µL of sample from each tube was used for measuring optical density (OD) at 820 nm.

For the estimation of total P, the entire seedling was first dried at 65°C in a hot air oven for 24 h. The next day, the dried plant material was finely ground into a powder, and 50 mg of powdered tissue was taken in a 100 mL hot test tube. Then, 1 mL of concentrated sulfuric acid was added to the tube, and the mixture was incubated overnight at RT. The following day, all tubes were incubated again at 120 °C for 2 h. Afterward, 1 mL of 30 % H_2O_2 was added to each hot tube, resulting in colourless solution. From each tube, 10 µL of the solution was transferred to new MCT and mixed with 600 µL of 0.42 % acidic ammonium molybdate and 100 µL of 10 % ascorbic acid. The mixture was properly mixed and incubated at 45 °C for 30 min. Then, 200 µL samples were taken from each tube, and the optical density (OD) was measured at 820 nm. The soluble Pi content was quantified using a standard curve for P [242].

Plants growth with ATP as sole P source

To check for the substrate affinity with ATP in *Slpap15*^{CR1} knock out lines, ATP was provided as the external source. For ATP treatment, 1 mM ATP was provided to plants grown in P deficient and sufficient conditions, as mentioned earlier. The treatment was given for five days and then the change in total P and soluble Pi content was calculated in both *slpap15* knock out lines and wild type by using the aforementioned assays of total soluble Pi content and total P. These assays were repeated independently at least three times.

5. Determination of acid phosphatase activity

Acid phosphatase activity of plant was assessed by SAP (secretory acid phosphatase activity) and also by using BCIP (5-bromo-4-chloro-3-indolyl-phosphate) staining for measuring root surface associated APase activity. For determination of SAP, roots were gently expelled from HP and LP media containing tomato seedlings. Immediately after wiping the extra water using blotting paper, the roots were submerged in a reaction buffer (10 mM MgCl2, 50 mM NaOAc, pH 4.9) containing 10 mM pNPP and incubated for at least 4h at 37°C. The reaction was terminated by adding 0.4N NaOH. Roots were now removed and absorbance was recorded for the yellow-colored solution at 410 nm. SAP activity was expressed in A410nm/plant/h. For visualization of the released pNPP from roots, we added the pNPP in ½ MS media and then incubated the roots on it for 20 min. Pictures of the roots were taken using high resolution camera [243].

To check the root surface associated APase activity, seedlings were taken out gently from media containing HP and LP solutions. Roots were washed multiple times with double distilled water (DDW). These roots were arranged vertically on transparent plates and soaked with blotting sheets to remove any extra water from them. These roots were immediately fixed by pouring 0.5 % agar solution containing 100 μM BCIP and incubated for 24h in the dark at RT. Images were taken by Canon high-definition camera [244].

6. In-gel assay was performed to check for total secretory APase activity

Total APase isoforms in root and shoot samples were detected separately using In-gel assay. The isozyme profile of APases is visualized by separating protein samples on SDS-PAGE (denaturing gel) or Native-PAGE (non-denaturing gel), which are then stained with the chromogenic substrates β-NAP and fast black K. For Native-PAGE analysis, SDS was omitted from the gel and the running buffer. HP and LP grown seedling root was taken out and grounded to extract the crude protein using protein extraction buffer (0.1 M KOAc, 20 mM $CaCl_2$, 0.1 mM PMSF, pH 5.5). Protein concentration was calculated using Bradfort assay reagent. 10 µl total protein in equal amount was taken and run in 12 % SDS PAGE gel. Gel was run first at 60 V for 30 min followed by 120 V for 150 min at 4 °C. Next, 50 ml reaction buffer (10 mM $MgCl_2$, 50 mM NaAc) was used to shake the gel 4-5 times in precooled reaction buffer. After this gel was incubated in 50 ml APase activity profiling detection buffer (0.3 mg/ml β-naphtyl acid phosphate, 0.5 mg/ml Fast black K, pH 4.9), prepared in the reaction buffer [243].

7. Phytase activity

Phytase activity was measured colorimetrically by monitoring the release of inorganic phosphate from phytic acid (Na-InsP6; Sigma-Aldrich). Phytase activity was measured using crude protein estimated by Bradford protein assay in seed, germinated seedling and roots separately. It is defined as the release of 1 mM of P per minute in Phytase assay buffer (1 mM phytate, 100 mM NaOAc, pH 4.5). Reaction was terminated by adding equal amount of 4 % TCA (trichloro acetic acid). The liberated inorganic orthophosphate was quantified using Ame's assay, as described earlier in MM section [245].

8. Time course expression kinetics and recovery experiment

The seeds were initially sprouted on absorbent paper. Subsequently, these young plants were transferred to standard ½ X Hoagland nutrient solution. After seven days of germination, they were subjected to a period of phosphate starvation lasting 15 days. During this time, samples were collected at various time intervals, starting from the first day of starvation (15 min, 4 h, 12 h, 24 h, 42 h, 72 h, 7 days, and 15 days). Additionally, the starved seedlings were later restored by switching from a low phosphate medium to a high phosphate medium, and samples were collected every hour. To preserve the samples, they were rapidly frozen using liquid nitrogen and stored in a deep freezer maintained at -80 °C.

9. Identification of PAPs in tomato

The protein sequences of Arabidopsis, rice, soybean PAPs were downloaded from sequence databases such as TAIR, Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/), and Soybase (https:// www.soybase.org/) and were used to generate an HMM profile of PAPs. The HMM profile was searched against the annotated protein sequence database of tomato (ITAG version 2.4) and putative PAP sequences were retrieved, as described previously [246]. The retrieved protein sequences were searched for the presence of metallophos domain using the SMART tool (http://smart.embl-heidelberg.de/) and five conserved motifs (DXG/GDXXY/GNH(D/E)/VXXH/GHXH) were searched manually using BioEdit (www.mbio.ncsu.edu/BioEdit/bioedit.html). The identified tomato PAPs were annotated based on their homology to AtPAPs, except SlPAP23, which was named based on its high homology with rice OsPAP23.

10. Analysis of gene structure and conserved motifs

Information on chromosomal localization, intron-exon organization, and coding sequence (CDS) of tomato PAPs were obtained from the SGN database. The amino acid composition, length, and their molecular weight were predicted using Gene runner (http://www.generunner. net/). The intron-exon organization was studied using GFF3 data (https://solgenomics.net/bulk/input.pl?mode=ftp), Plaza 4.0 [247] (https://bioinformatics.psb.ugent.be/plaza/versions/plaza_v4_dicots/), and GSDS server (http://gsds.cbi.pku.edu.cn/). Discovery of conserved motifs in the full-length protein sequences was made using the MEME Suite 5.1.1 with the following parameters: zero or one occurrence per sequence, with at least six motifs per protein sequence (https:// meme-suite.org/tools/meme). Subcellular localization was predicted using CELLO v2.5 (http://cello.life.nctu.edu.tw/). Signal P4.1 (http:// www.cbs.dtu.dk/services/SignalP/) was used to indicate the presence of any signal peptide. The 3-kb upstream region of all PAPs was used to manually screen the presence of cis-acting regulatory P1BS elements (PHR1 binding sites) (GNATATNC) in Gene runner (http://www. generunner.net/) **(Annexure 1)**. Map chart 2.30 tool (https://www.wur.nl/en/show/ Mapchart-2.30.html) was used for generating a chromosome localization map of tomato PAPs [248].

11. Phylogenetic analysis

The multiple alignments of tomato, *S. pennellii*, AtPAPs, and OsPAP genes were done using ClustalW [249]. The output '.aln' file was used as an input file in MEGA7 to generate an unrooted phylogenetic tree. The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model with 1000 iterations.

12. Estimation of total Anthocyanins

Anthocyanin pigments in seedlings grown under HP and LP condition were measured from the same node leaves. 250 mg of leaf tissue was weighed and crushed in 10 mL (methanol: water: HCl, 79:20:1, v/v/v) using mortar and pestle. The samples were incubated in dark for 2 h. Following incubation, the samples were centrifuged at 5000xg at room temperature, for 10 min. The absorbance of the supernatant was then measured at wavelengths 530 nm and 657 nm, as described previously. Total anthocyanins content was calculated as: $\{A_{530} - (A_{657})\}/gm\ FW$, where A_{530} and A_{657} are absorbance in nm of the solution. [250].

13. Histochemical analysis and quantification of ROS

The method described in https://bio-protocol.org/e1108#biaoti5025 was employed to carry out the detection of H_2O_2 and O_2^-. Leaf tissues that were collected and washed with DDW to eliminate unwanted substances. Leaves were arranged within a tissue culture plate containing six wells (Tarson, catalog number: 980010). To detect H_2O_2, leaf samples were immersed in a 1 mg/mL solution of 3,3'diaminobenzidine (DAB). For O_2^- detection, the samples were submerged in a buffer containing (2 % NBT prepared in 50 mM sodium phosphate). Plates containing NBT and DAB solutions were incubated overnight in dark at room temperature. On the following day, NBT/DAB solution was taken out and the leaf samples were subsequently destained to remove excess stain. It was done by boiling them in a solution containing acetic acid-glycerol-methanol (1:1:3) (v/v/v) at either RT for two days in rotor or at 100 °C for 5 min. Samples were stored finally in a glycerol-methanol solution (1:4) (v/v). The photos were taken where, H_2O_2 was observed as a brown color resulting from DAB polymerization while O_2^- appears blue in colour due to NBT. The final quantification was carried out using ImageJ software.

14. Homology modeling and docking

Tomato purple acid phosphatases (SlPAP15) protein sequence (UniProt ID A0A3Q7I9I9) [251,252] was used for homology modeling. Structural homolog templates for the query were retrieved from Protein Data Bank (PDB) [253] as predicted by the alignment tool BLASTP [254]. The N-terminal and C-terminal amino acid residues of the query sequence, not aligned to the template, were removed and Modeller v9.20 software [255], which uses spatial constrains satisfaction, was used for homology modeling. Truncation of the first 27 amino acids at N-terminal was made during the processing of the sequence for homology modeling. Two essential iron (Fe^{+3}) ions present in the template's active site were also incorporated in the model. A total of five models were generated, and these were filtered according to the DOPE score. These predicted models were then assessed to structure validations by using SAVES 5.0 (http://servicesn.mbi.ucla.edu/SAVES), ProSA (https:// prosa.services.came.sbg.ac.at/prosa.php), and QMEAN (https:// swissmodel.expasy.org/qmean/)[256][257][258]. The best-predicted model was selected for docking based on the DOPE and QMEAND scores and SAVES and ProSA structure validations.

Docking was performed using Schrodinger software [259]. The modeled protein structure was energy minimized at pH 5.5 with the OPLS3e force field using a protein-preparation wizard in Maestro. Different substrates, such as ATP, ADP, PEP, and IHP (phytate) were used as ligands. These molecules were extracted from PDB IDs 3X2V, 4XW6, 1FWN, and 3MMJ [260–262]. The Ligprep module was used for ligand preparation with the OPLS3e force field, pH 5.5, and a maximum one structure per ligand. The site containing metal ions (also active site), predicted by SiteMap, was selected for ligand docking. A grid of 15 Angstrom centered on the active site was generated using the receptor-grid generation module in Glide. Rotatable bonds across the grid (if any) were checked during grid generation. Finally, prepared ligands and protein with the grid were docked using the docking module with Extra precision (XP) criterion. These ligands were ranked based on the docking score.

15. RNA extraction and Qualitative real-time PCR

The extraction of total RNA from various treatments involving root and shoot, was carried out using the Favor-Prep™ Plant Total RNA Mini Kit (Favorgen Biotech Corporation, Taiwan), with a sample size of up to 100 mg of plant tissues. To ensure high-quality sequencing of RNA samples, Bionivid Technology Pvt Ltd (https://www.bionivid.com/) was entrusted with the RNA sequencing. The libraries were prepared and sequenced using the Illumina HiSEQ 4000 platform, employing parameters such as 150 bp paired-end sequencing and a minimum of 25 million reads per library. Rigorous quality control was performed on the paired-end sequence reads using the NGSQC Tool kit, selected reads with a Phred score >Q30 were taken up for further analysis. For reads alignment, the *Solanum lycopersicum* cDNA (ITAG 4.0) from SGN was utilized. Alignment was conducted using Kallisto quant, for the differential expression analysis DESeqR package was employed using default parameters. Transcripts exhibiting a $\log_2$ fold change above 1 and a p-value ≤ 0.05 were considered as differentially expressed genes in treated samples, in comparison to the signal values of their respective non-treated samples. The process of unsupervised hierarchical clustering was employed to analyze the differentially expressed genes. This clustering analysis was performed using Cluster 3.0, and the results were visualized using JavaTree Viewv1.1.6. To further enhance the visualization, heatmaps were generated using an online software tool known as Heatmapper (accessible at http://www.heatmapper.ca/). For annotation, all the differentially expressed transcript sequences were subjected to BLASTN against the Refseq Plant database to obtain Gene Ontology (GO) and KEGG Pathways information for the complete dataset. for significantly differentially expressed genes, Gene Ontology (Biological Processes, Cellular Components, and Molecular Functions) information were obtained from Ensemble Plant Biomart (SL3.0). Gene specific primers were designed using PRIMER plus version 2.0 for validation of differentially expressed genes using quantitative real-time PCR. Reactions were performed in 96-well plates designed for performing RT-qPCR using Bio-Rad CFX96 Touch Real-time PCR and Agilent Mx3000P QPCR systems. Normalization of obtained expression data was done using tomato GAPDH gene. Relative expression was quantified using $\Delta\Delta_{CT}$ method [263].

16. Construction of pSlPAP15: GUS transcriptional fusion construct

SlPAP15 harbor multiple P1BS (GNATATNC) elements, in 1-kb upstream promoter region. This region was amplified by PCR using gDNA of tomato as template. PCR product and binary vector pCAMBIA1391z was further purified and double digested with specific restriction enzymes. Ligation of was performed using vector and insert ratio of (1:6) with the help of T4DNA Ligase (Invitrogen Life Technologies, USA, catalog number: 5224017). The next day ligation mixture underwent transformation into *E. coli* cells. Transformants were grown on selective plates supplemented with 50 mg/L Kanamycin (Himedia, cataloge number: 25389-94-0), followed by colony PCR confirmation. Post PCR confirmation, plasmid was isolated from positive colonies and confirmed finally by Sanger sequencing. The confirmed plasmid was finally transformed via freeze thaw method into *Agrobacterium tumefaciens* strain

LB4404. The transformed colony was cultured, and glycerol stock was prepared for further experiments.

17. Generation of constructs for transient gene silencing

SGN database was used to examine the SlPAP15 gene features having gene locus ID Solyc09g091910. The genomic sequence was extracted from SGN database ITAG 3.20. The obtained exons were submitted to CRISPR-P 2.0 online tool (http://crispr.hzau.edu.cn/CRISPR2/) to find the best guide sequence. Parameters like NGG-PAM, U6 snoRNA promoter and exon sequence were considered as decisive parameters for the selection of gRNA. Guides were selected based on their high target potential, low off-target value and a free 5’ end in secondary structure. Restriction enzyme used for the cloning of gRNA is *Bsa*I, with the addition of *AtU6* promoter to the selected guide sequence (https://gatewayvectors.vib.be/collection/pen-2xatu6-template). PCR was employed to insert the dual guide and the complete fragment was purified as mentioned earlier. The destination vector pFASTRK-AtCas9-AtU6 (https://gatewayvectors.vib.be/collection/pfastrk-atcas9-atu6-scaffold) and the PCR purified fragments were double digested using *Eco*RI and *Pst*I, followed by ligation, transformation and final confirmation using colony PCR. Double digestion was performed using *Eco*RI and *Pst*I to confirm the cloning. Finally, the cloned fragment was confirmed using Sanger sequencing. Confirmed plasmids were transformed via freeze thaw, into AGL1 (agrobacterium competent cells) for generation of KO lines. Plant tissue culture was opted for generation of these lines, where the explants were prepared using the *cv* Pusa Ruby in 1/2 X MS solid media supplemented with kanamycin.

18. Generation of CRISPR/Cas9 knockout (KO) lines of SlPAP15

SGN database was used to examine the SlPAP15 gene features having gene ID Solyc09g091910. The exons obtained from the SGN database ITAG 3.20 were subjected to analysis using the CRISPR-P 2.0 online tool (http://crispr.hzau.edu.cn/CRISPR2/) to identify the optimal guide sequence. Parameters such as NGG-PAM and U6 snoRNA promoter and exon sequence were considered as decisive parameters. Guides were selected based on their high target potential, low off-target value and a free 5’ end in secondary structure. Restriction enzyme used for the cloning is *Bsa*I, with the addition of AtU6promoter to the selected guide sequence (https://gatewayvectors.vib.be/collection/pen-2xatu6-template). PCR was employed to insert the dual guide and the complete fragment was purified as mentioned earlier. The PCR-purified fragments and the destination vector pFASTRK-AtCas9-AtU6 (available at https://gatewayvectors.vib.be/collection/pfastrk-atcas9-atu6-scaffold) underwent double digestion using *Eco*RI and *Pst*I, followed by ligation, transformation and final confirmation using colony PCR and double digestion was done. Finally, the cloned fragment was confirmed using Sanger sequencing. Confirmed plasmids were transformed into AGL1 cells for generation of KO lines.

16.1 Generation of CRISPR Cas9 KO lines using plant tissue culture

Confirmed colonies are transformed into AGL1 and culture in YEM broth followed by incubation for 24 h at 28 °C with 180 rpm. 15 ml of YEM culture was inoculated with 1 % of primary culture for 18 h at 28 °C with 180 rpm. Cells were harvested once the OD 600 reached 0.5. Centrifugation was done at, 8000 g for 10 min at 20 °C. Pelleted cells were resuspended in 2 % MSO (20 g/L sucrose, 0.4 mg/L thiamine, 100 mg/L myo-inositol, 4.3 g/L MS salts). Final adjustment of number of cells was done to 1×10^8 cells/mL. Tomato seeds of cv Pusa Ruby were washed as earlier and rinsed at least for 10 times with ADDW under sterile conditions. Seeds were grown in dark for 2-days and then kept in light on 3rd day. Explants were prepared using the cotyledons and further incubated in KCMS (potassium containing MS) plates (sucrose 30 g/L, zeatin 0.1 mg/L, 2, 4-dichloro phenoxy acetic acid, thiamine 1.3 mg/L, myo-inositol 100 mg/L, 4.3 g/L MS salts, 200 mg/L KH_2PO_4, 0.8 % Agar, pH 6.0) in dark at 25 °C for two days. Two days post incubation, explants were treated with AGL1 resuspension culture for 10 min. Explants were soaked in sterile tissue paper and then transferred on KCMS/co-cultivation agar plates at 28 °C for 48 h. Shifting of explants was done in plant regeneration selective medium 2-ZKC (cefotaxime 500 mg/L , kanamycin 100 mg/L, zeatin 2 mg/L, sucrose 20 g/L, myo-inositol 100 mg/L, Nitsch vitamins 1 ml/L (1000 X), 4.3 g/L MS salts and 0.8 % agar) via placing the side facing upward that is adaxial. Explants performing better were sub-cultured to 2ZKC plates every week for three consecutive weeks. Finally, they are transferred to 1-ZKC plates (cefotaxime 500 mg/L, zeatin 1mg/L, kanamycin 100mg/L) bi-weekly. The process of transferring to selection plates continued until shoots emerged from the callus and possessed a minimum of one internode. Leaf samples were collected from developed calli in order to examine the incorporation of the Cas9 cassette. Cas9 confirmed plantlets were transferred to rooting media (sucrose 30 g/L, MS salts 4.3 g/L, Nitsch vitamins (1000X), agar 0.8 % and pH 6.0). Once root got emerged, then the plantlets were transferred to peat for three weeks for hardening and finally To lines were shifted to pots in green house.

19. Screening of lines for Cas9 cassette and Indels (insertion/deletion) presence in SlPAP15

Extraction of gDNA from the emerged leaf of calli and shoots was done by using Dellaporta method [264]. The isolated gDNA was confirmed in the Agarose gel and then used as a template for screening of Cas9-positive lines in T_0 generation. Primers were designed using the Gene runner tool to amplify 350 bp region upstream and downstream of target region within the gene. Reference sequence of wild type i.e Pusa Ruby gDNA was taken for alignment. Amplified fragment from both wild type and the expected edited line was sent for Sanger sequencing. Analysis of sequences were done using ICE online tool (Synthego- CRISPR performance analysis). The confirmed plants were carefully taken to pots in the greenhouse and maintained till the seed were harvested. The confirmed lines in each generation were again screened for the presence of Cas9 and indels as mentioned above finally at T_3 generation for Cas9-ve line and also the presence of Indels in them by amplification of exon sequences, containing indel followed by sanger sequencing. The confirmed lines were checked finally for any off-targets by PCR amplification of the off target in WT and Cas9-ve transgenic line, followed by sanger sequencing. Simultaneously, lines were transferred to pots in the greenhouse and maintained for harvesting T_1 generation seeds. The independent lines T_1 seeds were grown in ½ MS-agar supplemented with kanamycin (100 mg/L) agar bottles. Wild-type seeds were used as a negative control in this selection strategy. After four weeks, gDNA was isolated from the leaves of the plantlets and transferred to coconut peat, followed by their transfer to the greenhouse after another week. Plants were grown further to T_2 generation in greenhouse, and confirmed for Cas9 negative lines.

20. Screening of the KO lines for presence of any off-targets

Potential off-target list obtained while choosing guides in CRISPR-P 2.0 online tool were analyzed. Off-target guides situated in the CDS of other genes were selected for screening. Further, the selected SlPAP15 guide was used as BLAST query in the Sol genomics database to screen for any other off-targets in the tomato genome. Primers ***were*** designed for all the target genes with hit in the CDS region as mentioned above and fragments were PCR amplified, purified and sent for Sanger sequencing for any off-targets editing.

21. Generation and histochemical staining of pSlPAP15: GUS Arabidopsis lines

The WT Col-0 and Arabidopsis homozygous SlPAP15 stable promoter line seeds were surface sterilized. 70 % ethanol was used for sterilization with proper stirring for two min, with subsequent treatment with 4 % sodium hypochlorite for 10 min in a laminar air flow. Seeds washing was done multiple times with ADDW and placed in agar plates. The plates with the were kept at 4 °C in dark (wrapped with aluminum foil) for three days. Subsequently, the plates were transferred to culture room with a temperature of 22 °C and a light-dark cycle of 16/8 h. Three days post incubation, seedlings were moved to ½ MS-Agar plates containing high or low phosphate and then grown for the next 15 days. Careful attention was given to avoid damaging the roots while removing the seedlings. For histochemical staining, the seedlings that were washed with DDW and placed in a GUS solution prepared in phosphate buffer 50 mM (pH 7.2) containing, $K_3Fe(CN)_6$ (5 mM), $K_4Fe(CN)_6$ (5 mM), Triton X-100 0.2 % (v/v), and X-Gluc (2 mM). The seedlings were then incubated overnight at 37 ºC with gentle shaking. To remove the chlorophyll, leaves were de-stained using 70-95 % ethanol solutions. Finally, images of the seedlings were captured using light microscope.

22. Transactivation of SlPAP15 promoters by SlPHR1/SlPHL1 in *N. benthamiana*: pCAMBIA1391z: SlPAP15 with pCAMBIA1302:SlPHR1/L1

The pre-transformed recombinants in agrobacteria were used for infiltration for transactivation assay as described earlier by K. Norkunas et al. (2018) [265]. In brief, YEM plates were prepared containing kanamycin (50 mg/L) and rifampicin (30 mg/L). Plates were inoculated with single colony for each construct and incubated for 24 h at 28 °C. The subsequent secondary cultures were maintained in YEM supplemented with earlier mentioned kanamycin and rifampicin antibiotics and 200 µM Acetosyringone. Cultures were grown for 24h at 28 °C. The next day, bacterial cultures were centrifuged and the supernatant was discarded. The pellet was resuspended in buffer [MES (10 mM), $MgCl_2$ (10 mM), Acetosyringone (200 µM)] to get OD_{600} of 1. Cultures were incubated for 2 h and infiltrated in upper leaf of 6-8-week-old plants with different plasmids mixed in a 1:1:1 ratio (pCAMBIA1391z-SlPAP15: GUS: pCAMBIA2302:PHR1/L1: p19) or (pCAMBIA1391z-GUS: pCAMBIA2302:PHR1/L1: p19). Afterwards, plants were at first kept in dark for 24 h and subsequently in a culture room at 22 °C with a relative humidity of 65 % and aforementioned light intensity and photoperiod cycle for next 72 h.

23. Generation of Over-expression line of *SlPAP15*

Data pertaining to *SlPAP15* gene was analysed in the SGN, using gene ID, Solyc09g091910, and the coding sequence were extracted from ITAG 3.20 in SGN database. The coding sequence was amplified using cDNA, and then amplified product was purified using the kit as mentioned above. pBI121 expression vector was used for generation of overexpression lines. Double digestion of both the vector and purified PCR product was done using restriction enzymes *Xba*I and *Sac*I at 37 °C for 30 min. Further ligation was kept for overnight at 16 °C using T_4DNA ligase enzyme, followed by transformation, colony PCR confirmation and double digestion. The cloned product was confirmed again via sequencing and finally transformed in *Agrobacterium* AGL-1 cells. Lab optimized stable tomato transformation protocol was used to generate the overexpression lines. In total 10 overexpression lines were generated.

24. Western Blot analysis

500 mg of fresh leaf tissue was pulverized for the crude protein using pre-chilled motor and pestle. The pulverized tissue was resuspended in pre-chilled protein extraction buffer solution (100 mM Tris-base pH 7.5, 10 % glycerol, 100 mM Na_2-EDTA, 2 mM EGTA, 2 mM PMSF and 1X-protease arrest) and incubated on ice for 30 min [266]. The samples were followed by centrifuging at 20,000 RCF (relative centrifugal force) for 30 min at 4 °C and the supernatant was collected. Quantification of the crude protein was done using Bradford reagent, while BSA (Bovine serum albumin) was used for plotting the standard curve [267]. Then, 10 µg protein was taken for western blot analysis to check the protein levels in SlPAP26b silenced and non-silenced seedlings. In brief, 10 µg of protein of all the samples were run on 15 % SDS-PAGE, and protein was further transferred by semi-dry blotting onto PVDF membrane (Immun-Blot, Bio-Rad, USA) for 1½ h in 25 mM Tris-base, 190 mM glycine and 20 % methanol. The blots were washed 4-5 times at 10 min intervals with 1X TBST buffer (50 mM Tris pH 7.5, 150 mM NaCl, 0.1 % Tween 20) and then the blots were blocked with 5 % BSA at 4°C overnight. Further, the blots were washed as described above and incubated for 1h with 1/5000 dilution SlSPX2 polyclonal antibody (generated in rabbits against the synthesized peptide: CEPKRSVSSGDGDDVRASKR by Abgenex Pvt. Ltd. India). Unbounded antibodies were washed away with 1X TBST buffer as described above, and then blots were incubated in 1/20000 dilution of peroxidase-conjugated Goat anti-Rabbit IgG (Abgenex Pvt. Ltd.) for 1h. Further, the blots were washed thoroughly with 1X TBST buffer, as described earlier. The visualization of the specific protein in blots was done by adding the equal ratio mixture of luminol and peroxide solution on the blots, and images were developed using Clarity western ECL substrate in ChemiDoc XRS+ system (Bio-Rad USA) [266].

25. Virus-induced gene silencing method (VIGS)

Tomato seedlings were grown for a period of seven days, as mentioned in the preceding sections. To suppress the expression of candidate genes, namely *SlPAP15*, *SlPAP17b*, *SlPAP26a* and *SlPAP26b*, the VIGS was employed as demonstrated by M. Senthil Kumar et al. (2013)[268]. In summary, culture of constructs was kept in YEM broth media with Rifampicin at 30 mg/L and Kanamycin at 50 mg/L, followed by incubation at 28°C overnight. On the following day, the secondary culture was introduced into Induction Media (IM) comprising kanamycin at 50 mg/L, rifampicin at 30 mg/L, and Acetosyringone at a concentration of 200 µM, and incubated at 28 °C for 24 h. The cells were then collected by centrifugation at 3000xg for 10 min, and resuspended gently using an equal volume to that of the original culture medium (10 mM $MgCl_2$, 10 mM MES at pH 5.5). Once more, centrifugation was performed at 3000xg for 10 min, and subsequently, the cells were resuspended in a buffer containing $MgCl_2$ (10 mM), MES (10 mM) at pH 5.5, using half the volume of the secondary culture. The final mixture was prepared using (1) pTRV1 and pTRV2-*SlPAP15*/*SlPAP17b*/*SlPAP26a*/*SlPAP26b* (2) pTRV1 and pTRV2-*SlPDS* (3) pTRV1 and pTRV2. The mixture was mixed in 1:1 ratio, resulting in final optical density (O.D.) of 0.15 at 600 nm. The final cultures were then infiltrated into both cotyledons using 1 mL syringe. These seedlings were grown in culture room maintained at 22 °C, 65 % relative humidity, and a light intensity of 200 $\mu mol/m^2/s^{-1}$, with a photoperiodic cycle of 16 h light and 8 h dark, for a period of seven days. On the next day, they were subdivided into two groups containing HP (1.25 mM) and LP (5 µM, LP) media for the next 15 days. Post termination the VIGS plants were tested for the presence of viral coat-protein (Cp) gene. Primer specific to Cp was made and a standard PCR reaction was kept to check for the amplification in silenced seedlings. All the Cp confirmed plants were utilized for RNA isolation and morpho-physiology and biochemical study.

26. Plant growth condition for AM symbiosis study

For the AM fungi root colonization experiments, tomato (*Solanum lycopersicum* cv. Pusa Ruby) seeds were sterilized as described earlier. For mycorrhizae treatment, pots were filled with autoclaved sand and mixed with VAMCARE (a biostimulant containing *Glomus* species, purchased from GENUINE FERT & PEST PVT. LTD., Bangalore, India; https://farmerscastle.com/product/vam/), as described by us previously, Srivastava et al. (2021)[242]. In each pot, 5 g of VAMCARE containing 300 AM spores was mixed with 400 g sand. Inoculation of heat-killed mycorrhizae acted as the control for the AM fungi experiments. Tomato seeds were germinated and then transferred to pots containing half-strength Hoagland's media and grown for 14 days in a culture room. The seedlings were then transferred to autoclaved sand

(400 g/per pot) in the presence of active or heat-killed mycorrhizae. The experiment was performed with 10-15 seedlings per set. Half-strength Hoagland's media (200 µM P) was provided to the growing plants twice a week. Control and AM fungi-treated pots were placed in separate trays to avoid cross-contamination. Plants were grown for 21 days in the same culture room and then harvested for further assays. During the experiments, the position of each pot was arbitrarily changed to avoid any position effect.

27. Optimization of mycorrhizae and phosphate concentration

For mycorrhizae treatment, five different concentration was taken (1 g, 2 g, 5 g, 10 g, 20 g; 1 gm = 50 spores) and was added to 400g sand after mixing. Autoclaved sand (400 g with heat killed mycorrhizae) alone in each pot was used as a control. Nutrient solutions with four different concentrations of Pi (50 µM, 100 µM, 200 µM, 50 µM, 1.25 mM) was administered to the sand twice a week. The final Pi concentration used for the experiment is 200 µM, which is supplemented weekly twice in sand containing 5 g (optimized mycorrhizae concentration). To work under natural conditions 400 g of red soil was taken and mixed with 5 g of mycorrhizae. Plants were grown for 4 weeks and then harvested for further assays.

28. Histochemical staining of roots

28.1. Pelikan Ink blue staining

The AM fungi colonization of roots was confirmed by Pelikan ink blue staining method [269]. After harvesting, the roots were washed with DDW to remove the tanned area. Further, 10 % KOH was used for clearing the remaining tanned region at 90 °C for 15-20 min. The roots were washed again with double distilled water and stained using Pelikan blue ink-vinegar staining reagent. The treated roots were left to stain for 2-3 min, followed by destaining using concentrated glacial acetic acid. The stained roots were checked to confirm the extent of AM fungi colonization under a light microscope (Leica M105FC, Germany) at 40X magnification.

28.2. Uvitex-2B staining

For Uvitex-2B staining we have used the same procedure as mentioned above till washing. Further, 10 % KOH was used to clear the tanned region at 90 °C for 2 h. Roots were incubated at RT for 10 min. Roots were rinsed in water and incubated in Uvitex-2B (0.05 %) at 90 °C for 120 min. Roots were kept in water overnight to eliminate the excess Uvitex2B. Slides were prepared the next day and microscopy was performed. Two independent acquisitions were performed, one at 405/420–480 (excitation/emission) for Uvitex2B and one at 488/530 LP for auto-fluorescence, with pinhole sizes of 102 and 100 µm, respectively [270].

29. Mycorrhizal growth response (MGR) and Mycorrhizal effectivity ration (MER)

To calculate MGR, whole plant was at first taken out from sand and then the roots were washed out using running water multiple times until the tanned area was cleaned. Then the roots were soaked in tissue paper for a while and then the plant was taken for drying overnight in hot air oven at 60 °C. Plants were taken out next day and the dry weight was measured in weighing balance. The mycorrhizal growth response (MGR) was calculated using the dry weight of AM and NM (non-mycorrhizal) seedlings using the formula (AM-NM)/ (NM) X 100 [271]. While for MER, mainly the total leaf area was measured using Image J software and then (AM (total leaf area) -NM (total leaf area)/ (NM (total leaf area) X 100 was used to calculate mycorrhizae effectivity ratio [272].

30. PUE (Phosphorus use efficiency) and PAE (Phosphorus acquisition efficiency)

PUE is defined as the amount of biomass produced per unit of absorbed P. First total dry weight was measured using weighing balance. Then total P was calculated from whole plant as described earlier. Now, for each plant PUE was measured and the average was taken to check the changes observed in plant grown under phosphate deficient and sufficient conditions. Same was also measured in case of mycorrhizae treated plants [273]. While for PAE, it relates to the different extents to which plants are able to mobilize phosphorus from poorly soluble sources. Mean PAE (%) = mean [phosphate contents in shoot under phosphate-deficient conditions/phosphate contents under normal phosphate supply (1.25 mm KH_2PO_4) × 100][274]. While for Mycorrhizae samples PAE was calculated as the amount of P taken up by roots expressed in µg P/mg root DW.

Result

In this study, I first aimed to catalog tomato seedlings' response to low Pi conditions at morpho-physiological and biochemical levels in the selected Indian and Bulgarian tomato accessions and identify low Pi sensitive and tolerant germplasm. Then, I studied genome-wide changes at transcript levels to identify the candidate genes regulating P starvation responses (PSR) in the seedlings of tolerant and sensitive varieties. Subsequently, I carried out the functional characterization of selected promising PSI candidates to unravel their roles in tomato seedlings. Due to the lack of knowledge on the functions of plant PAPs during AM colonization, I also characterized tomato *PAP20*, a non-PSI/Mycorrhizae responsive PAP, for its role in mycorrhization. Altogether, the primary objective of this study was to identify the tolerant and sensitive accessions and functionally characterize promising PSI candidate genes, which could be used later to bioengineer tomato varieties with improved Pi-acquisition or Pi-use efficiency.

Objective 1:

1. Screening of tomato germplasm under Pi starvation for identifying tolerant and sensitive accessions

In this objective, physio-morphological and biochemical responses of Indian and Bulgarian genotypes were screened under prolonged Pi starvation at a 15-D time point to identify tolerant and sensitive varieties. In total, 34 tomato genotypes were screened. Morpho-physiological parameters such as lateral root number, root hair number and density, total soluble Pi levels, total anthocyanins content and total P content were investigated to screen tolerant and sensitive varieties. Overall, the tolerant varieties showed less severe effects of Pi starvation **(Fig. 5)**. The tolerant varieties exhibited higher photosynthetic efficiency (measured using Fv/Fm and PS II) than the sensitive ones. Seedlings dry weight and PUE (Phosphorus use efficiency) was found to be higher in tolerant varieties as they grew better under Pi deficiency than their sensitive counterparts. The tolerant varieties also accumulated lower levels of total anthocyanins than the sensitive accessions **(Fig. 6A)**. Total P content and total soluble Pi in the categorized tolerant and sensitive varieties showed varied results. For example, Zlatista, categorized as a sensitive variety in our study, accumulated higher P and soluble Pi content than most of the tolerant varieties **(Fig. 6A)**. However, we did not find any significant difference in total chlorophylls and total carbohydrates levels between the tolerant and sensitive varieties **(Fig. 7)**. Based on the initial screening of tomato accessions, we categorized AV (Arka Vikas) and PR (Pusa

Ruby) as susceptible varieties, and AVai (Arka Vaibhav) and CLN as tolerant varieties **(Fig. 8A)**.

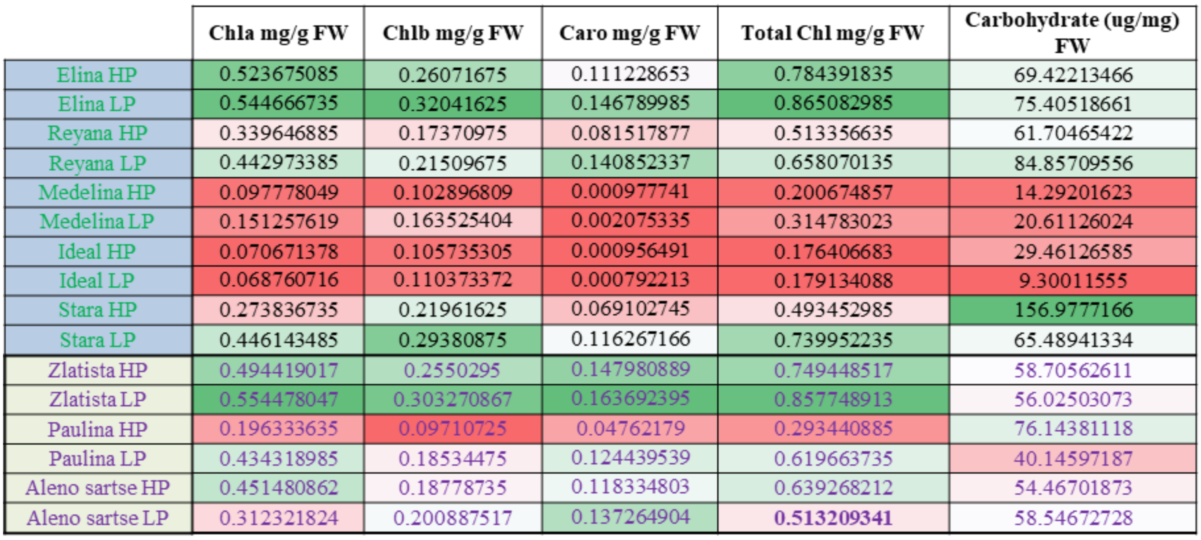
__Fig 5: Screening of tomato germplasm under Pi starvation.__ Tomato seedlings phenotypes of the selected tolerant and sensitive varieties grown under HP and LP conditions. The seedlings of sensitive varieties showed severely inhibited shoot growth. HP = High Phosphate, LP = Low Phosphate.

(A)

	Fv/Fm	PSII	Total anthocyanins (A530nm/mg) FW	Root DW (g)	Shoot DW (g)	Total DW (g)
Elina HP	0.7914	0.6115	0.421406203	0.032	1.006	1.038
Elina LP	0.589	0.4349	0.821561161	0.0758	0.645	0.7208
Reyana HP	0.7604	0.5989	0.244068428	0.1085	1.35	1.4585
Reyana LP	0.7598	0.5781	0.424945316	0.066	1.235	1.301
Medelina HP	0.8086	0.6208	0.348448399	0.0776	1.59	1.6676
Medelina LP	0.7269	0.5464	0.925431217	0.060333333	1.05	1.110333333
Ideal HP	0.795444444	0.605222222	0.16650361	0.0396	1.098	1.1376
Ideal LP	0.7023	0.5131	0.721540836	0.10775	1.1742	1.28195
Stara HP	0.8091	0.6275	0.14847577	0.0612	0.9	0.9612
Stara LP	0.7372	0.5274	0.450148328	0.0505	0.686	0.7365
Zlatista HP	0.802	0.627	0.9627	0.0125	0.172	0.1845
Zlatista LP	0.626	0.429909091	3.3653	0.00782	0.0415	0.04932
Paulina HP	0.7881	0.6174	[illegible]	0.0361	0.8903	0.9264
Paulina LP	0.6052	0.4409	2.603934678	0.025	0.2839	0.3089
Aleno sartse HP	0.758454545	0.614727273	0.6329	0.02	0.16425	0.18425
Aleno sartse LP	[illegible]	0.117616164	1.7784	[illegible]	[illegible]	[illegible]

	Total soluble Pi	PUE (Biomass / mg P)	Total P (mg/g)
Elina HP	16.3084977	0.029011227	23.9148799
Elina LP	1.894480544	0.112385214	5.438437857
Reyana HP	13.342396	0.040612782	18.73794319
Reyana LP	2.322985836	0.093608192	6.20565347
Medelina HP	19.86099707	0.061007864	27.33418091
Medelina LP	1.357597684	0.077334398	14.35756098
Ideal HP	12.03359191	0.036535893	31.13650422
Ideal LP	1.269120102	0.012449832	10.29692573
Stara HP	13.64740617	0.022520482	21.32281195
Stara LP	1.673457354	0.051857823	7.069328765
Zlatista HP	22.38	[illegible]	[illegible]
Zlatista LP	3.95	[illegible]	11.22283106
Paulina HP	8.679693231	0.037467036	18.06921678
Paulina LP	[illegible]	[illegible]	[illegible]
Aleno sartse HP	12.8	[illegible]	[illegible]
Aleno sartse LP	4.111	[illegible]	11.70341504

***Fig 6: Physiological and biochemical analyses of selected tolerant and sensitive varieties grown under HP and LP conditions**. Higher photosynthetic efficiency Fv/Fm, PSII, total dry weight (DW), Phosphorus use efficiency (PUE) and lower total anthocyanins accumulation was observed in tolerant varieties. Total soluble Pi content and total P content were measured in all the candidate tolerant and sensitive genotypes. DAT = Day after treatment.*



	Chla mg/g FW	Chlb mg/g FW	Caro mg/g FW	Total Chl mg/g FW	Carbohydrate (ug/mg) FW
Elina HP	0.523675085	0.26071675	0.111228653	0.784391835	69.42213466
Elina LP	0.544666735	0.32041625	0.146789985	0.865082985	75.40518661
Reyana HP	0.339646885	0.17370975	0.081517877	0.513356635	61.70465422
Reyana LP	0.442973385	0.21509675	0.140852337	0.658070135	84.85709556
Medelina HP	0.097778049	0.102896809	0.000977741	0.200674857	14.29201623
Medelina LP	0.151257619	0.163525404	0.002075335	0.314783023	20.61126024
Ideal HP	0.070671378	0.105735305	0.000956491	0.176406683	29.46126585
Ideal LP	0.068760716	0.110373372	0.000792213	0.179134088	9.30011555
Stara HP	0.273836735	0.21961625	0.069102745	0.493452985	156.9777166
Stara LP	0.446143485	0.29380875	0.116267166	0.739952235	65.48941334
Zlatista HP	0.494419017	0.2550295	0.147980889	0.749448517	58.70562611
Zlatista LP	0.554478047	0.303270867	0.163692395	0.857748913	56.02503073
Paulina HP	0.196333635	0.09710725	0.04762179	0.293440885	76.14381118
Paulina LP	0.434318985	0.18534475	0.124439539	0.619663735	40.14597187
Aleno sartse HP	0.451480862	0.18778735	0.118334803	0.639268212	54.46701873
Aleno sartse LP	0.312321824	0.200887517	0.137264904	**0.513209341**	58.54672728

***Fig 7:** Analysis of total Chlorophyll a, Chlorophyll b, total chlorophylls, total carotenoids and total carbohydrates content in tolerant and sensitive varieties.*

The detailed re-analysis of the selected tolerant (AVai and CLN) and sensitive varieties (AV and PR) confirmed higher leaf biomass and fresh weight in the tolerant accessions under LP condition **(Fig. 8B) (Fig. 9C)**. Interestingly, the tolerant varieties accumulated lower total Pi levels than the sensitive variety under the LP condition, while such change was also noticed under HP condition, when PR was compared with CLN. **(Fig. 9D) (Fig. 8F)**.

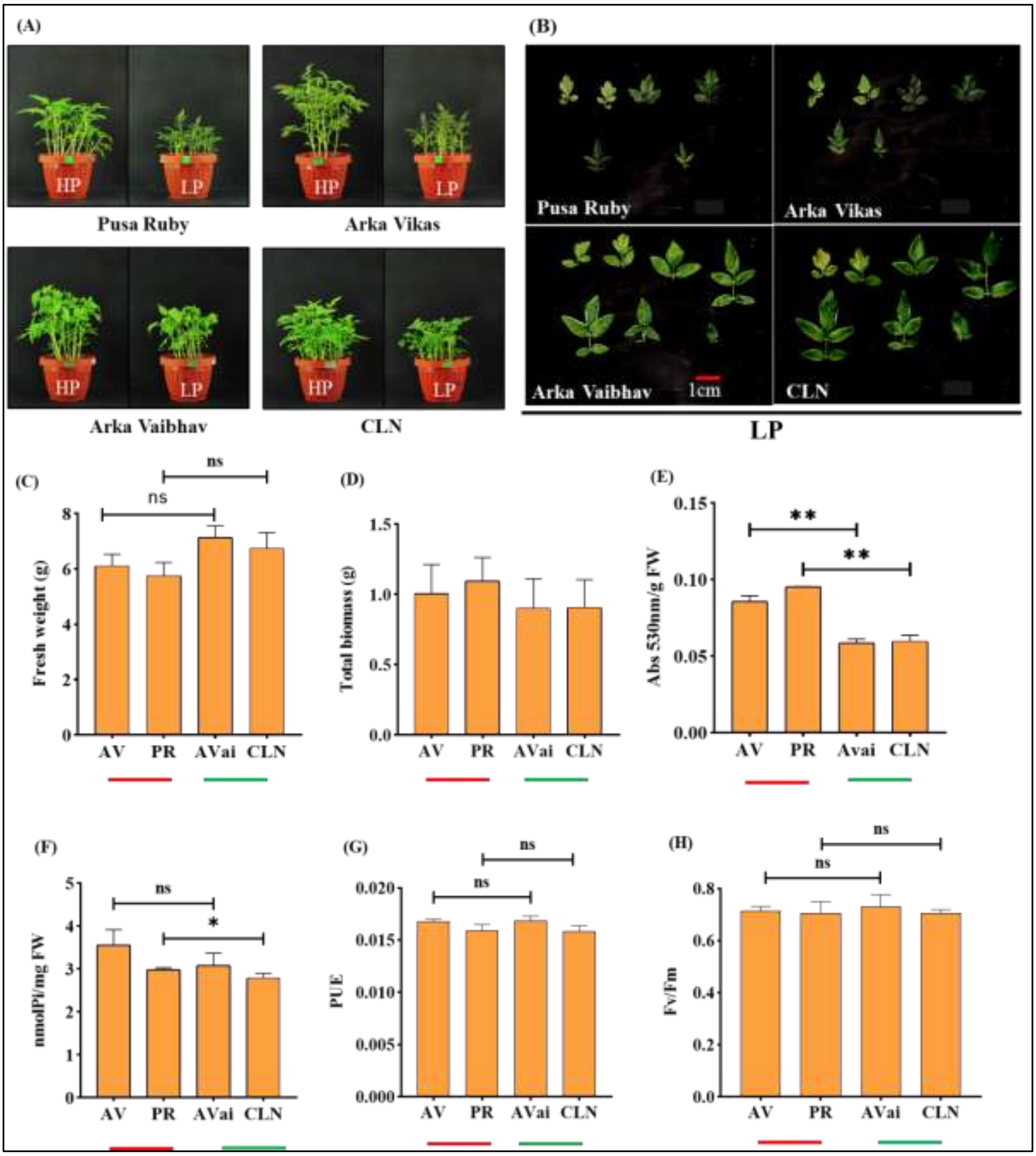


Fig 8: Physio-biochemical characterization of tolerant and sensitive genotypes under HP condition (A) *Shoot phenotype.* ***(B)*** *Leaf morphology.* ***(C-D)*** *Fresh weight and total biomass.* ***(E-F)*** *Total anthocyanins content and total soluble Pi content.* ***(G)*** *PUE (phosphorus use efficiency).* ***(H)*** *Photosynthetic efficiency**.** The red bar at the bottom of the graphs indicates*

sensitive varieties and the green bar is used for tolerant varieties. The experiment was repeated at least twice.

We also checked the earlier-mentioned parameters for in-depth analysis. We observed higher biomass, PUE, and photosynthetic efficiency in tolerant genotypes under LP conditions. Under HP condition, we did not notice any such change except for total soluble Pi content. The Pi content was found to be reduced in Avai, a tolerant variety **(Fig. 8D, G, H) (Fig. 9B, E, F)**. Surprisingly, almost negligible anthocyanins accumulation was observed in tolerant genotypes under HP and LP conditions **(Fig. 8E) (Fig. 9C)**. We also observed lesser root hairs and decreased lateral root density in the tolerant varieties than in sensitive varieties under LP conditions **(Fig. 9G-I)**. We also investigated reactive oxygen levels (ROS) in these varieties. ROS generated under phosphate stress can disrupt cellular redox balance, leading to a compromised antioxidant defense system. NBT/DAB staining and subsequent quantification showed lower accumulation of O^{2-} and H_2O_2 in the leaves of tolerant varieties than in sensitive varieties **(Fig. 10A-D)**. Owing to the peroxidase activity associated with some PAPs, we next checked the root surface associated APase activity (SAP) and intracellular APase activity (IAP) in both tolerant and sensitive varieties. This analysis showed significantly lower APase activity in tolerant than in sensitive varieties **(Fig. 10E, F)**. In search of the probable genes responsible for the reduced APase activity, we then checked the expression of root preferential PSI SlPAP genes (*SlPAP1*, *SlPAP9b*, *SlPAP10b*, *SlPAP15*, *SlPAP17b*, and *SlPAP26b)*. Interestingly, milder induction in the mRNA levels of *SlPAP10b* and *SlPAP15* was observed in tolerant varieties than in the sensitive ones **(Fig. 11A-F)**.

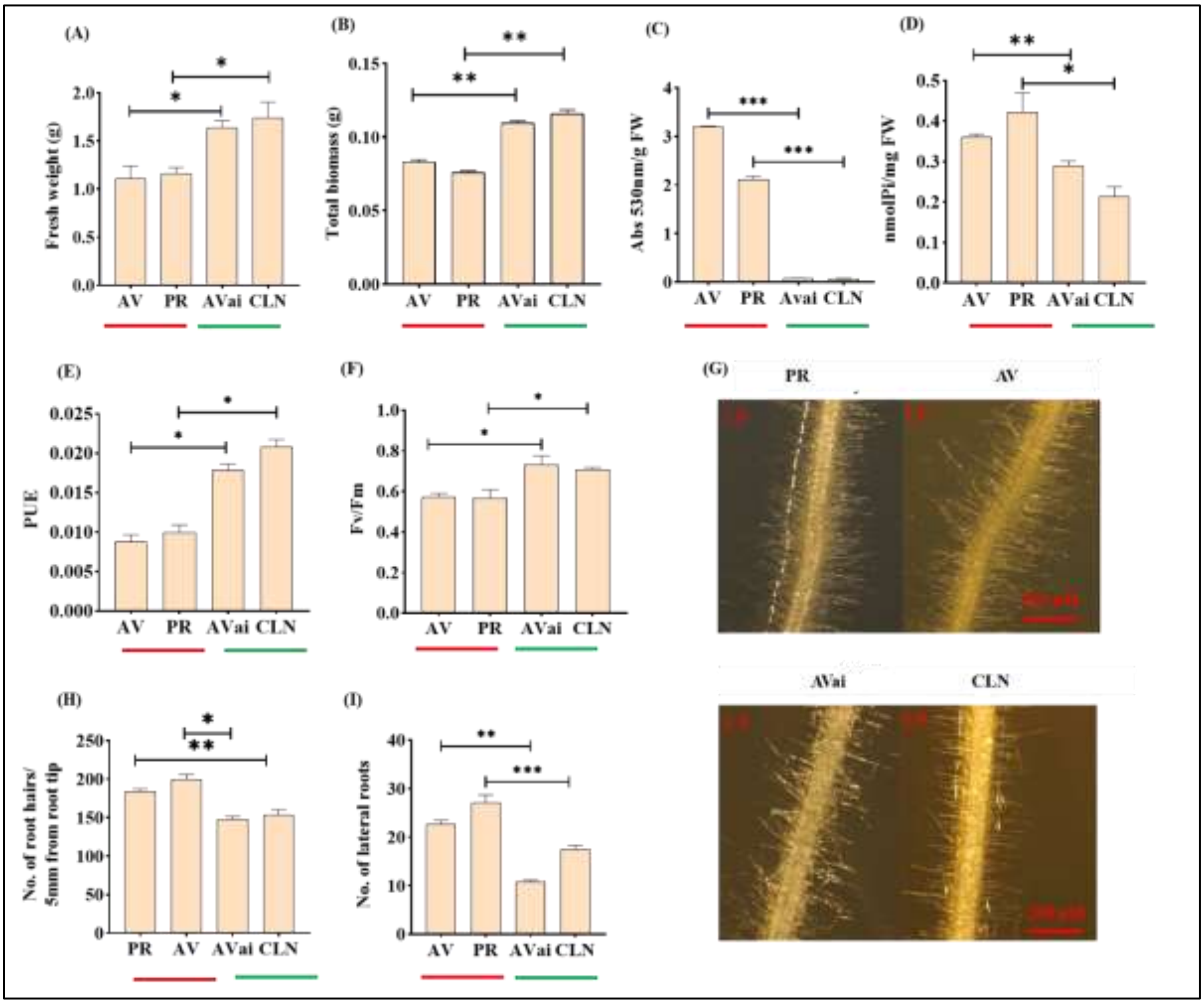


Fig 9: Physio-biochemical characterization of tolerant and sensitive genotypes under LP conditions (A-B) *Fresh weight and total biomass.* ***(C-D)*** *Total anthocyanins content and total soluble Pi content.* ***(E)*** *PUE (phosphorus use efficiency).* ***(F)*** *Photosynthetic efficiency****. (G)*** *Representative image of root hairs in tomato genotypes screened for change in RSA****. (H-I)*** *Total number of root hairs and lateral roots****.*** *N = 10, (N= Total number of roots checked from each experiment).*

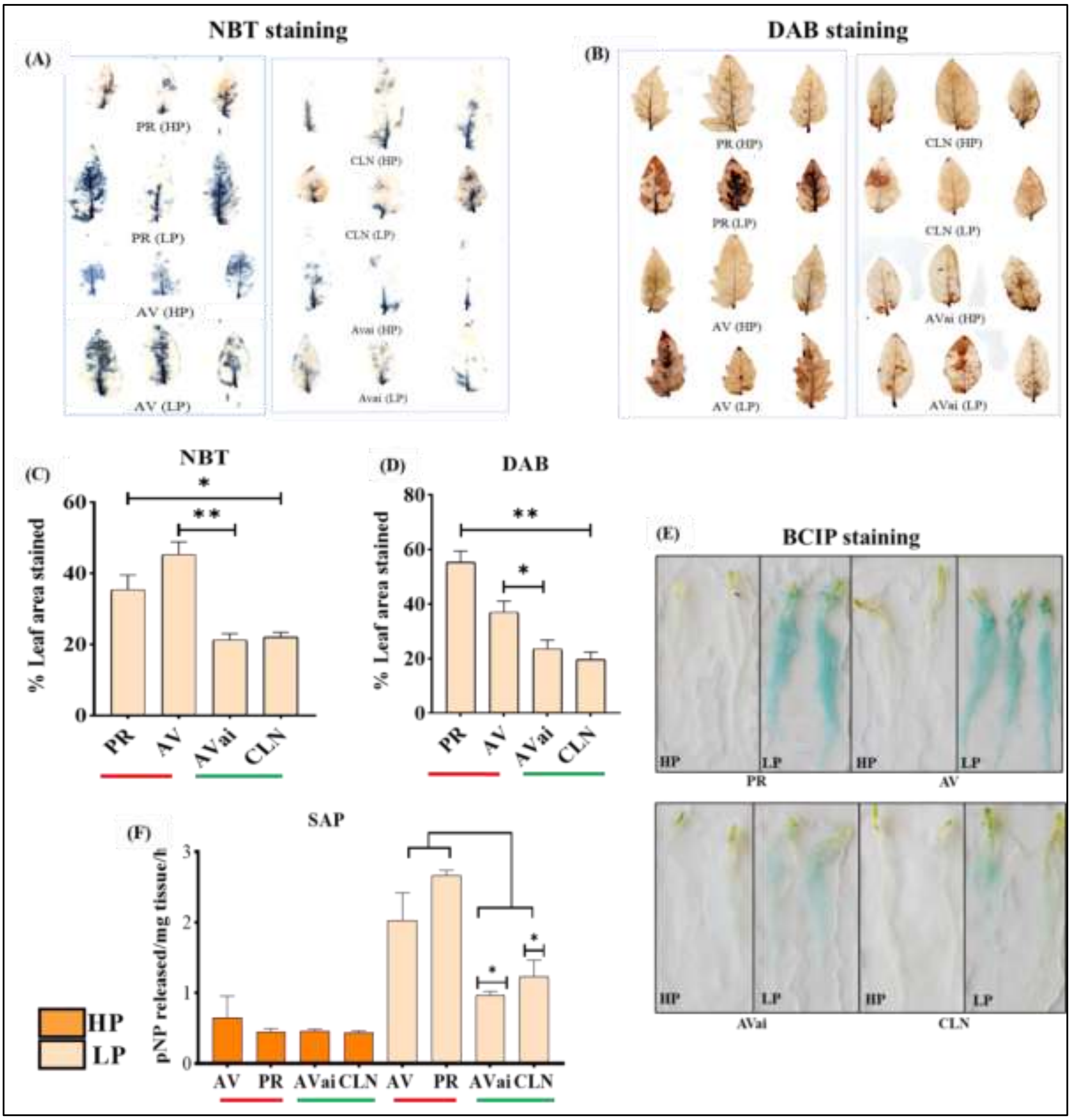


Fig 10: ROS levels and APase activity in tolerant and sensitive genotypes under HP and LP conditions *(A-B)* *Histochemical staining of leaves from the 4th node using NBT/DAB.* ***(C-D)*** *Quantification of generated ROS using ImageJ software.* ***(E)*** *Histochemical staining of root surface associated APase activity using BCIP.* ***(F)*** *Quantification of SAP activity using pNPP assay.*

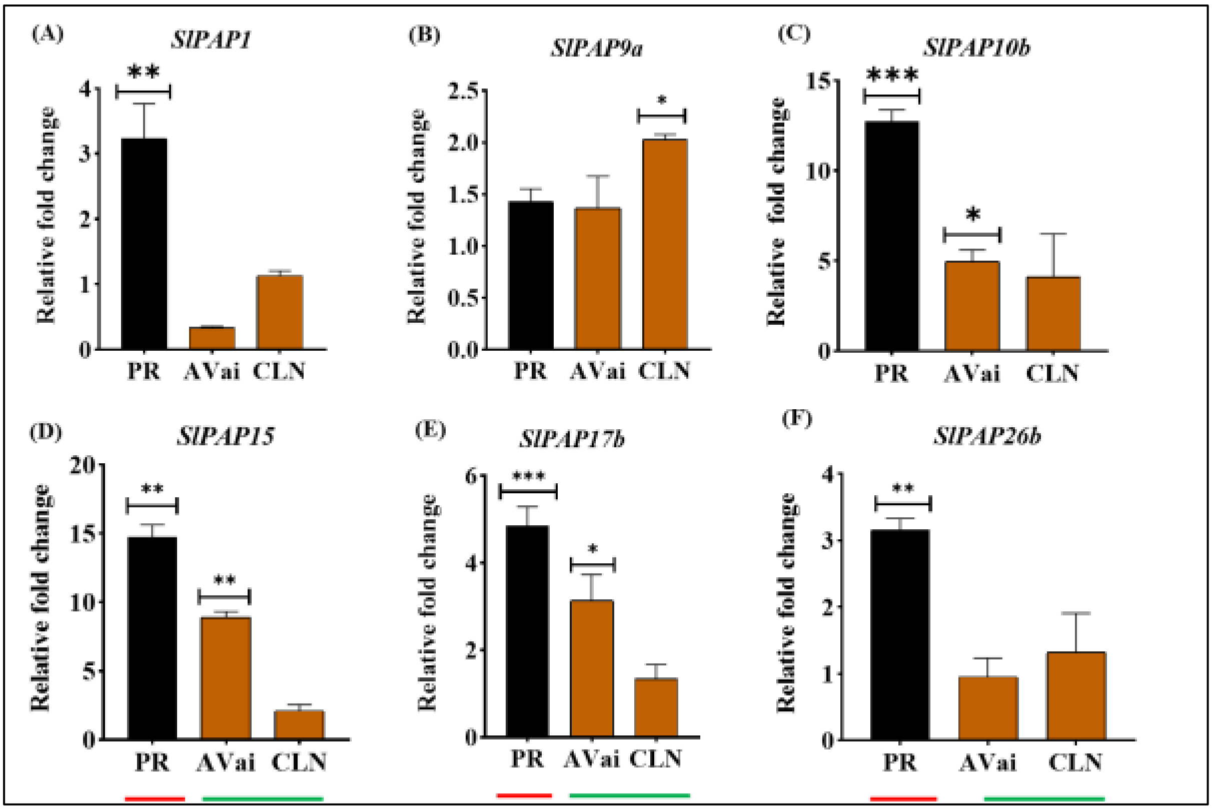
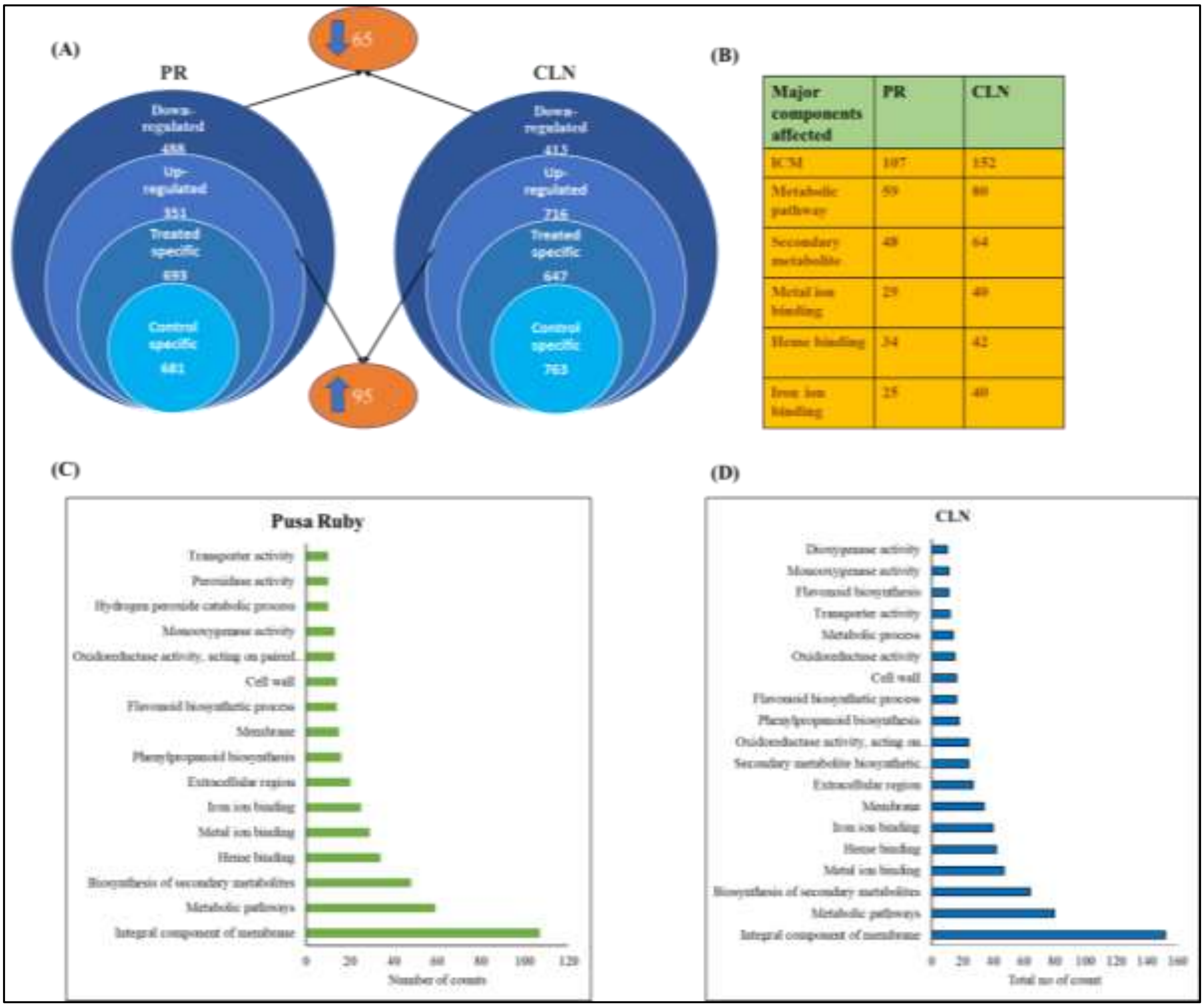


***Fig 11: Differential expression of SlPAPs in the candidate genotypes (A-F)** Transcript profiling of selected PAPs (SlPAP1, SlPAP9a, SlPAP10b, SlPAP15, SlPAP17b and SlPAP26b) in the roots of tolerant (CLN, AVai) and sensitive (PR, AV) genotypes upon 15-DAT of Pi starvation.*

Transcriptome analysis of a tolerant (CLN) and sensitive variety (PR)

To capture the genome-wide transcriptional changes, we studied transcriptomes of PR and CLN tomato seedlings to identify commonly and distinctly induced phosphorous starvation inducible (PSI) genes at 15-D of starvation in the two genetic backgrounds. Transcriptome analysis revealed more upregulated than down-regulated genes in the CLN background **(Fig. 12A)**. A total of 716 transcripts were upregulated (FC ≥2-fold, p-value 0.05), whereas 413 transcripts were downregulated (FC ≤ 2-fold, p-value-0.05) in the CLN. In contrast, 351 transcripts were upregulated, and 488 transcripts were downregulated in PR, the sensitive variety. A set of 95 transcripts were identified as the commonly upregulated genes in PR and CLN, while 65 transcripts were commonly downregulated in both genetic backgrounds **(Fig. 12A-D)**. Gene ontology analysis of differentially expressed genes (DEGs) revealed enrichment of vacuole and endoplasmic reticulum-related functions under 'cellular component' and hydrolase activity under "molecular function' categories exclusively in both tolerant and sensitive varieties **(Fig. 13A-H)**. This analysis identified promising PSI candidates from different categories:

transcription factors, acid phosphatases, transporters, lipid remodeling, and ROS. Genes encoding enzymes belonging to the peroxidase class were found to be affected more in PR, while genes belonging to transporters, F-box, and MYBs were enriched as DEGs in CLN**) (Annexure 6, 7).**

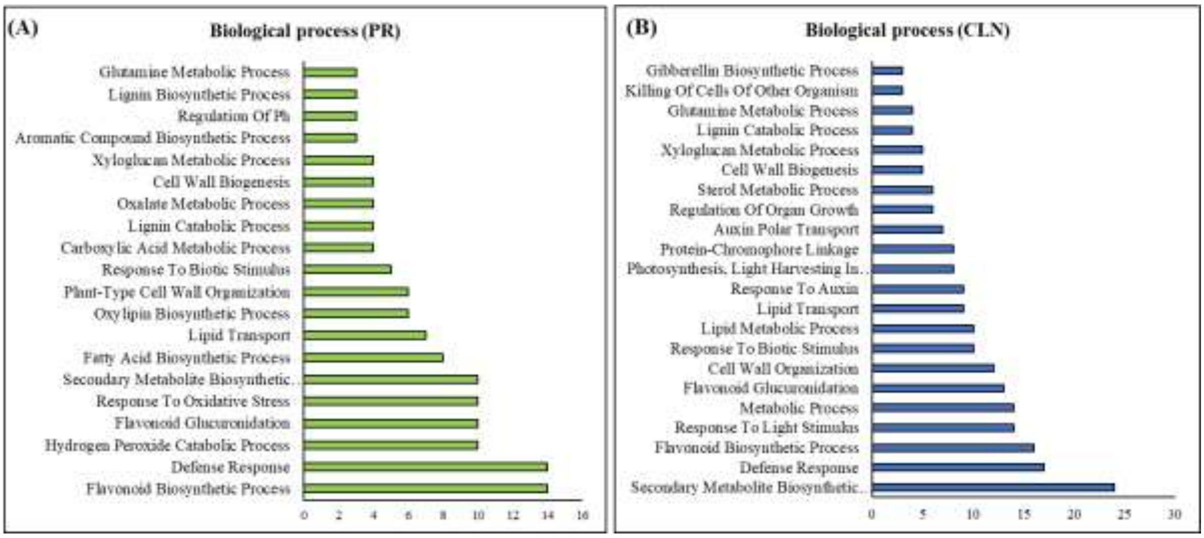


Major components affected	PR	CLN
ICM	107	152
Metabolic pathway	59	80
Secondary metabolite	48	64
Metal ion binding	29	49
Heme binding	34	42
Iron ion binding	25	40

Fig 12: Number of differentially and commonly expressed genes in tolerant and sensitive genotypes (A) *Venn diagram indicating differentially expressed genes in PR and CLN.* ***(B-D)*** *Gene ontology-based number of components affected in various categories such as integral component of membrane, metabolic pathways, secondary metabolites, heme-biding, metal ion binding, oxidoreductases class of enzymes in PR and CLN.*

1.1.1. Pi starvation activates the transcription of several transporters in CLN and PR

Scrutiny of the differentially expressed genes for GO and KEGG pathways revealed differential regulation of many genes with transporter activity. In total, 27 transcripts responding to different transporters were differentially expressed in CLN. These transporters are sub-categorized into inorganic ion transporters and sugar homeostasis. Among inorganic ion transporters, three sulfate transporters (Solyc04g054730, Solyc04g072760, and Solyc09g082550), one amino acid (Solyc03g013440), one high-affinity nitrate Solyc03g112090, and one ammonium transporters3-like, (Solyc09g065740) were differentially expressed. Among the carbohydrate's transporters, higher transcript levels of genes involved in bi-directional sugar transporters (Solyc03g097570, Solyc04g064630, Solyc05g024260, Solyc03g007360, and Solyc01g099880), glycerol-3-phosphate transporter (Solyc06g060910) and UDP-galactose/glucose transporter (Solyc08g080270) were observed in CLN. In the sensitive variety, the total number of transporters in the differentially regulated genes was lesser than in the CLN. Some of the transporters detected in PR are the highly expressed sugar transporter (ERD6-like) Solyc02g085170 and two bi-directional sugar transporters, N3-like (Solyc05g024260) and (SWEET5) Solyc03g114200.



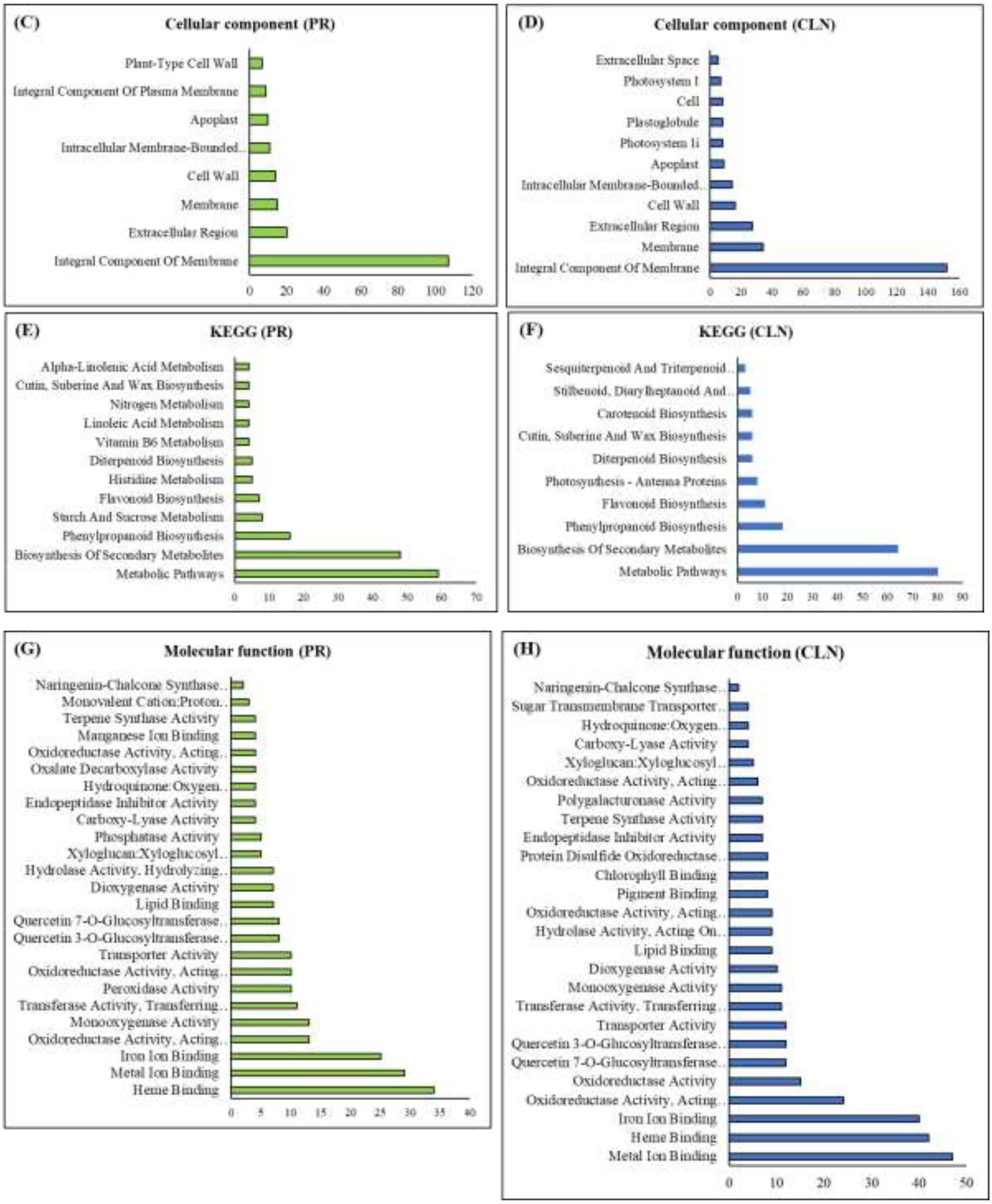


Fig 13: In-depth exploration of biological insights into the differentially expressed genes. (A-H) *Different biological processes, molecular functions, cellular components and KEGG pathways at 15-DAT in PR and CLN LP seedlings.*

1.1.2. Pi starvation differentially alters the expression of many transcription factors in both genetic backgrounds

A group of proteins that contain approximately 135-380 amino acids conserved SPX domain are involved in maintaining intracellular inorganic phosphate (Pi) homeostasis in plants and fungi. PHOSPHATE STARVATION RESPONSE 1 (PHR1) and its homologs are Myb-CC type transcription factors (TFs) that are the primary positive regulators of PSR in plants. In PR, the higher accumulation of (*SlSPX1)* Solyc08g060920, (*SlSPX2)* Solyc12g009480 and (*SlSPX3)* Solyc01g090890 transcripts were detected when compared to that of the tolerant variety. A SlPHR1-like 3 gene was also present in the differentially expressed genes in PR, while no differentially expressed SlPHR was detected in the tolerant variety. The most prominent categories in this set of genes belonged to "MYB" transcription factors, which were found to be in the tolerant variety. Some of the important MYBs in this list include Solyc10g086250 (MYB75), Solyc10g086270 (MYB113), Solyc05g053150 (MYB24), Solyc08g076700 (MYB53). In sensitive variety, Solyc01g094360 (MYB41), Solyc08g076700 (MYB53), Solyc11g072060 (MYB97), and Solyc04g005600 (MYB106) were differentially expressed. Among the downregulated ones, PHL5-like (Solyc10g078720) was commonly downregulated in both varieties. Besides TF encoding genes, transcripts of purple acid phosphatases such as Solyc03g098010 (*SlPAP17b),* Solyc04g016490 (*SlPAP16)*, Solyc07g008570 (*SlPAP27c*), and Solyc04g008245 (*SlPAP8)* were found to be at higher levels in PR.

Objective 2:

2. Identification and functional characterization of Pi starvation inducible purple acid phosphatase genes for their roles in Pi acquisition and homeostasis.

Purple acid phosphatases (SlPAPs) are the enzymes that hydrolyze organic phosphate into inorganic phosphate and liberate Pi. The activity of these enzymes is enhanced in plant tissues under Pi deficiency. While these enzymes are well-studied in many plant species, the complete range of their functions in tomato remains poorly explored. This objective identified PSI SlPAPs for their role in tomato PSR. An inquiry into the tomato genome identified 25 SlPAP members. These genes were named based on their sequence similarity with Arabidopsis homologs. Based on the predicted size of the identified proteins, 18 tomato PAP members belonged to the large purple acid phosphatase category. Of these, 16 tomato PAPs retained all three domains, including pur_ac_phosphN, metallophos and metallophos_C, whereas two SlPAPs, SlPAP25a, and SlPAP27a, contained only two domains **(Table 3)**. On the contrary, only six members, including SlPAP4, SlPAP7, SlPAP17a, SlPAP17b, SlPAP23b, and SlPAP24a, have only metallophos domain and are grouped into the small PAP category. SlPAP23a harboured only a pur_ac_phosphN domain. Examination of their gene structure showed varied exons number from 2 (SlPAP9a; SpePAP9a) to 13 (SlPAP9b) **(Fig. 15C)**. The molecular mass of the identified PAP members ranged from 25.7 kDa (SlPAP24a) to 73 kDa (SlPAP9a). Prediction of their subcellular localization disclosed that while SlPAP9a and SlPAP17a are cytoplasmic, SlPAP7 and SlPAP23a are likely localized at the plasma membrane. The remaining SlPAP members were predicted to be either secretory and/or lysosomal in their localization **(Table 3)**. At least three groups, including SlPAP10a:b, SlPAP23a:b, and SlPAP27a:b:c located on chromosomes 1, 4 and 7,

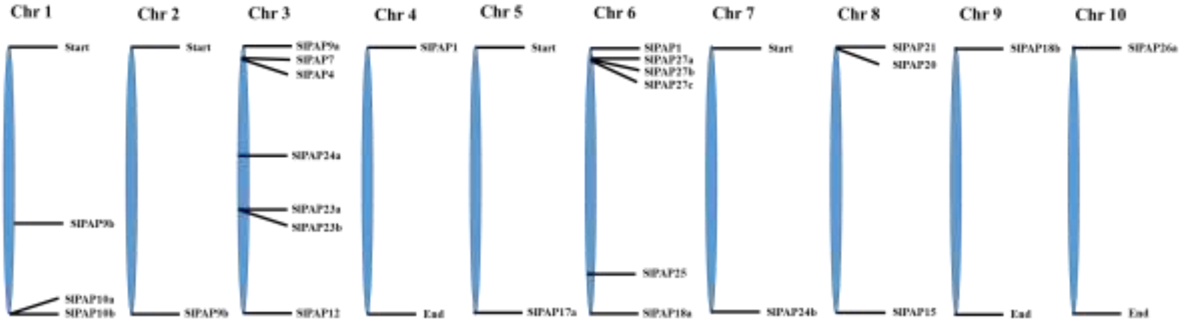


***Fig 14. Chromosome localization of SlPAPs using MAPchart 2.30 tool**. These genes are non-uniformly distributed in 10 chromosomes. Chromosomes 2 and 11 did not harbour any SlPAP member.*

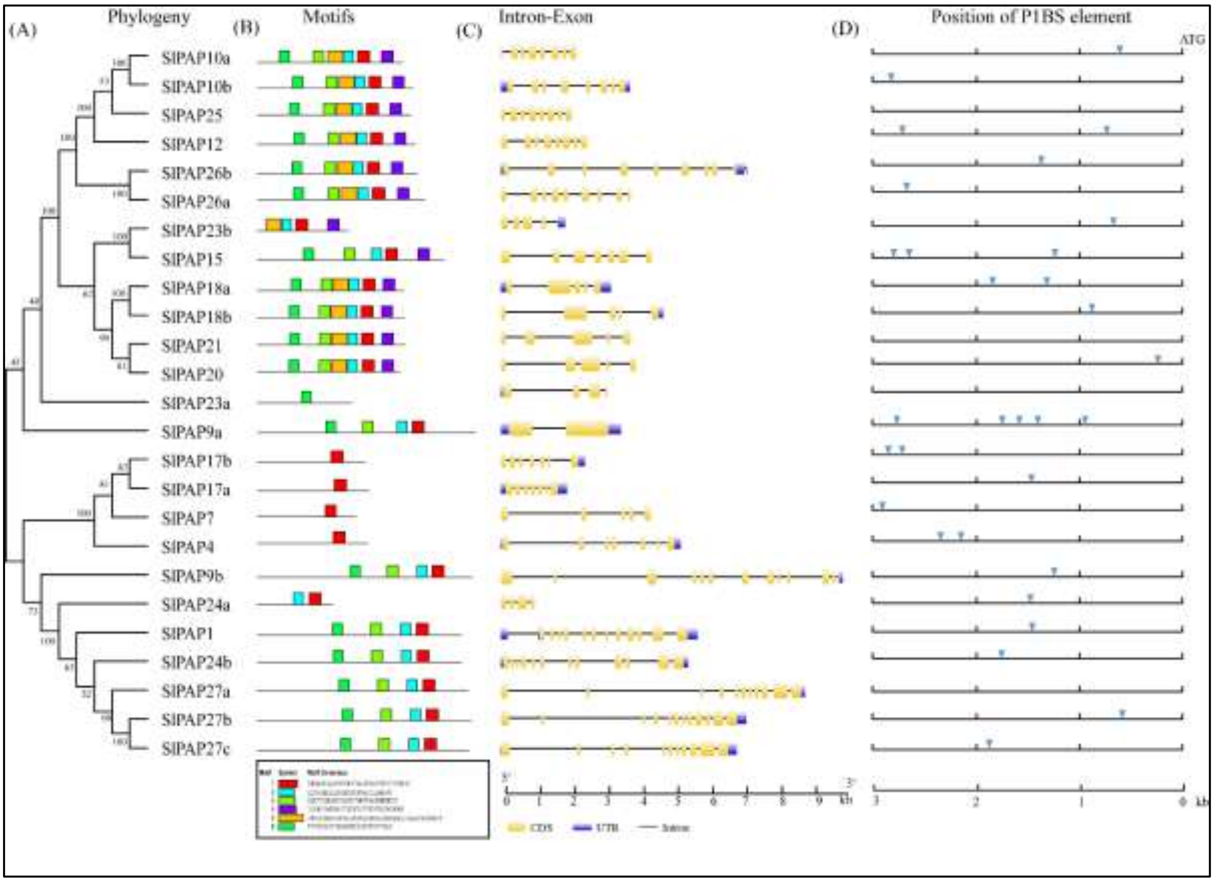


Fig 15: Gene structure and motif analysis of the identified tomato SlPAPs. (A) *Phylogeny of tomato SlPAPs. Multiple alignments of the sequences were performed using ClustalW, and the Neighbor-Joining (NJ) tree was constructed using MEGA7.* ***(B)*** *Conserved motif analysis of the identified SlPAP protein sequences was done using the MEME Suite 5.1.1 with the following parameters: zero or one occurrence per sequence, with at least 6 motifs per protein sequence.* ***(C)*** *The intron-exon arrangement of the genomic loci of tomato SlPAPs was made using GSDS2.0.* ***(D)*** *Arrangement of P1BS cis-regulatory elements in the 3-kb upstream promoter region of the identified SlPAP.*

respectively, appears to be tandemly duplicated. Further, based on the location of gene pairs, namely SlPAP9a:b; SlPAP17a:b, SlPAP18a:b, SlPAP24a:b, and SlPAP26a:b on different chromosomes, these genes seem to have undergone segmental duplication event **(Fig. 14)**. Further,18 SlPAPs were found to have all the five conserved blocks with conserved motifs containing characteristics seven metal-binding residues **(Fig. 15B)**. At the same time, the remaining SlPAPs lacked one or more conserved blocks. For instance, SlPAP7 lacks the fifth block (GHXH), whereas SlPAP15, SlPAP27a, SlPAP27b, and SlPAP27c lack the fourth block (VXXH). Similarly, SlPAP23a did not contain the third (GNH(D/E) and fifth (GHXH) blocks **(Annexure 2)**. Further examination of the N-terminal glycosylation sites predicted a variation in the number of such predicted sites in SlPAPs. While SlPAP18a and SlPAP20 lacked any glycosylation site, SlPAP1, SlPAP9b, SlPAP10a, SlPAP10b, SlPAP15, SlPAP24b, SlPAP25, SlPAP27b, and SlPAP27c were predicted to possess at least five or more glycosylation sites **(Annexure 4)**. The remaining SlPAPs were predicted to have less than five such sites. The N-terminal transmembrane helix analysis showed that while 15 SlPAP members lacked this structural feature, the remaining ten members possessed only one such domain **(Annexure 4)**.

2.1. Prediction of structural elements guiding substrate specificity of tomato SlPAPs

In a recent study, Feder et al. (2020)[68], through homology modeling crystallographic data, substrate docking simulations, and phylogenetic analysis, reported several active sites that define substrate specificities of plant SlPAPs. To investigate the conservation of the identified residues in the newly identified SlPAP members, we performed multiple sequence alignments of the selected AtPAPs and all SlPAPs. The relative position of the conserved residues is based on their place in the complete amino acid sequence of SlPAP15. The analysis showed high conservation in the amino acid residues asparagine (R283) and histidine (R284). In contrast, the other residues positioned at R337, R338, R374, R375, and the REKA domain spanning from R433-R437 showed less conservation and varied among the identified SlPAP members **(Fig. 17)**. Nonetheless, it remained conserved in Arabidopsis, tomato, and *S. pennellii,* AtPAP12, SlPAP15, and SPePAP26 homologs. These residues are considered necessary for substrate binding, irrespective of the identity of the reactant, among tomato and Arabidopsis homologs. Among the proteins used in this alignment, the atypical REKA domain was found only in AtPAP15, SlPAP15, and SpePAP15. Other SlPAP members showed the presence of variants of this motif. Besides REKA, the lysine residue at R337 is also vital for binding to phytate. This residue is conserved in seven tomato SlPAPs, including SlPAP15, SlPAP26a, and SlPAP26b **(Fig. 17)**.

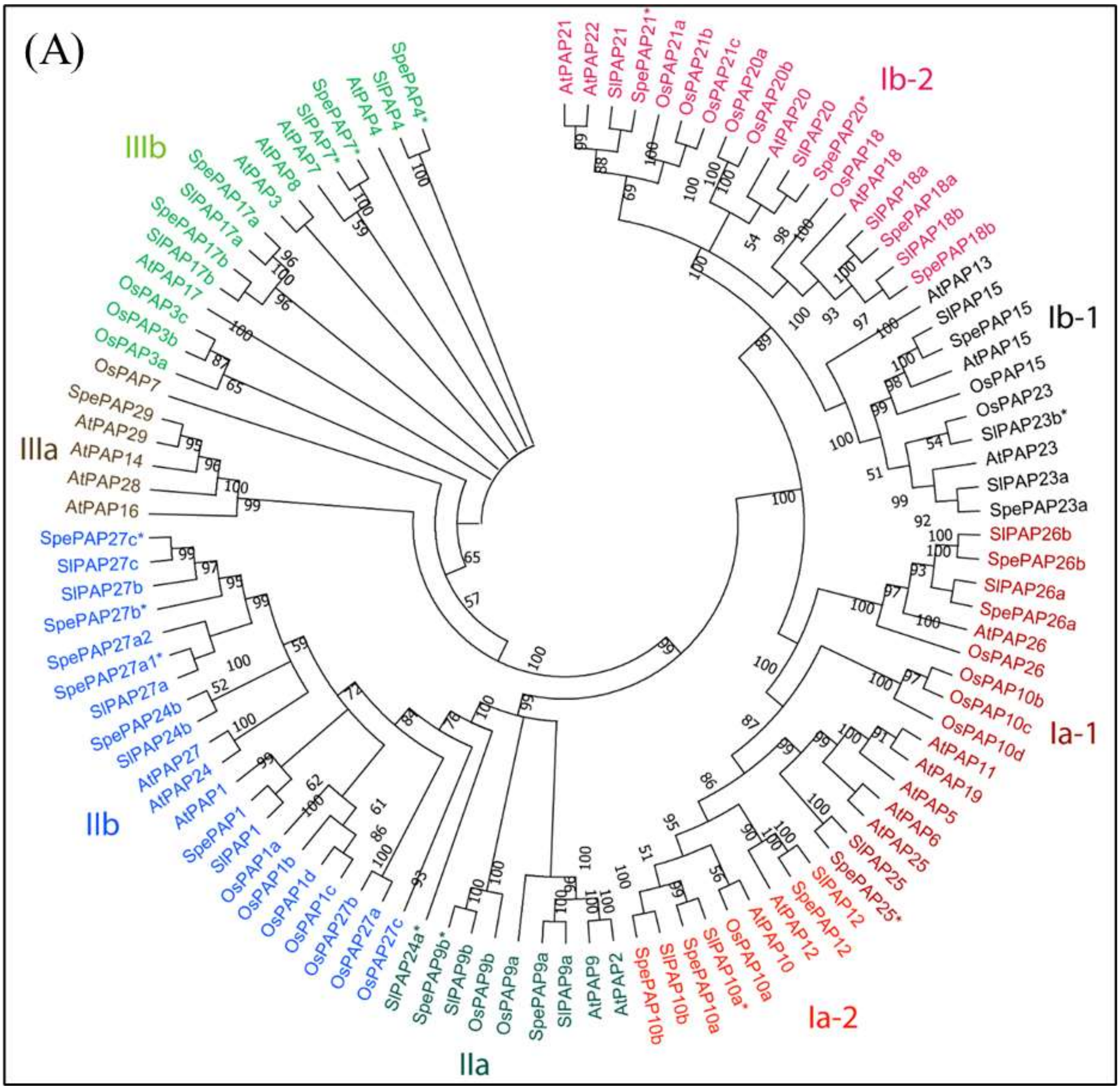


Fig 16: Phylogenetic analysis of Arabidopsis, rice, tomato, and S. pennellii PAP homologs*. *(A) *The amino acid sequences of the identified homologs were aligned using ClustalW, and the Neighbor-Joining (NJ) tree was constructed using MEGA7. The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model with 1000 iterations. Values with >50 % supporting the node are indicated. Members of different SlPAP subfamilies are represented in different colors.*

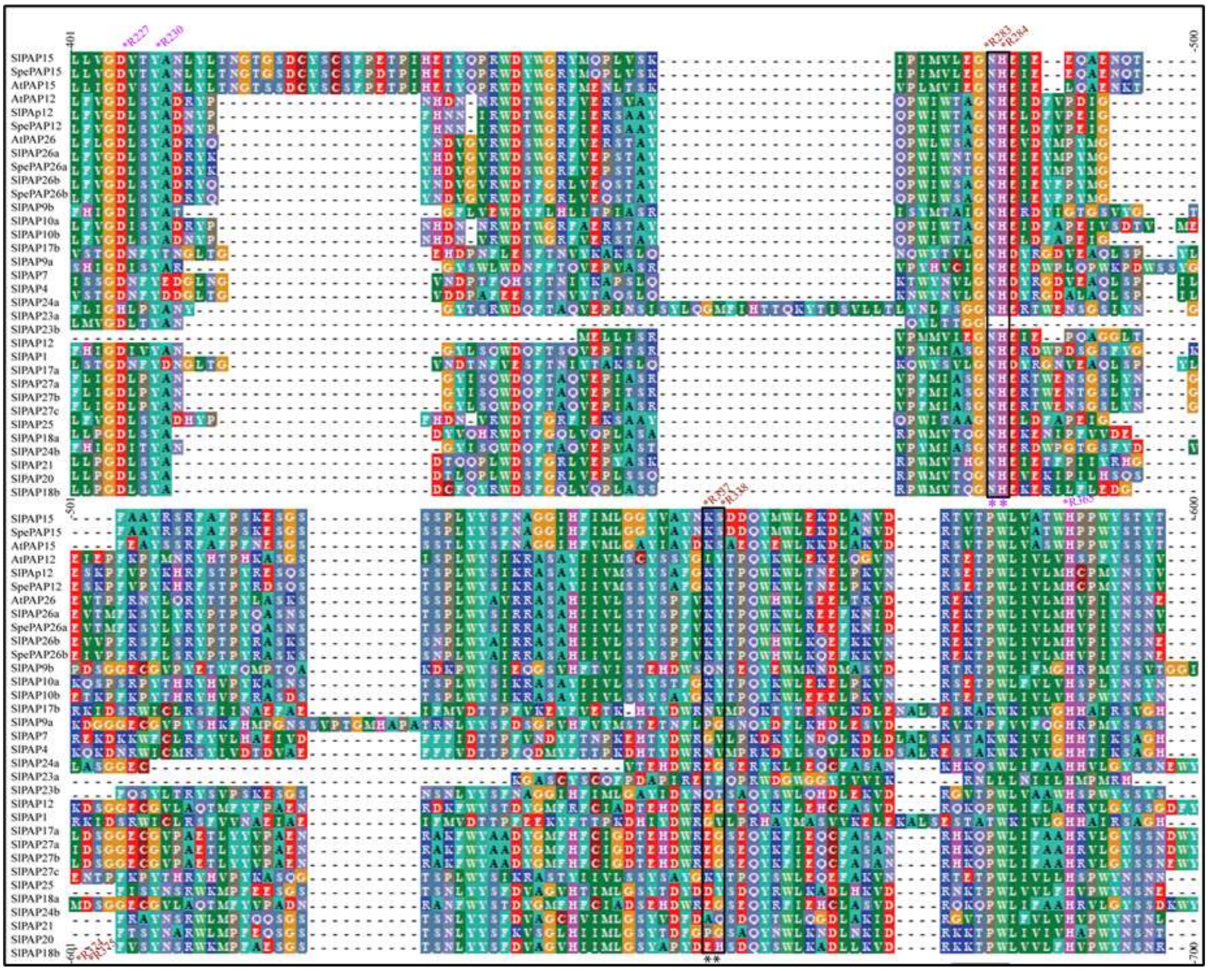


*Fig 17: **Alignment of selected AtPAPs, SpePAP, and all SlPAPs**. The alignment was done using ClustalW in BioEdit.*

Table 3. Characteristic features of tomato purple acid phosphatase (SlPAP) genes.

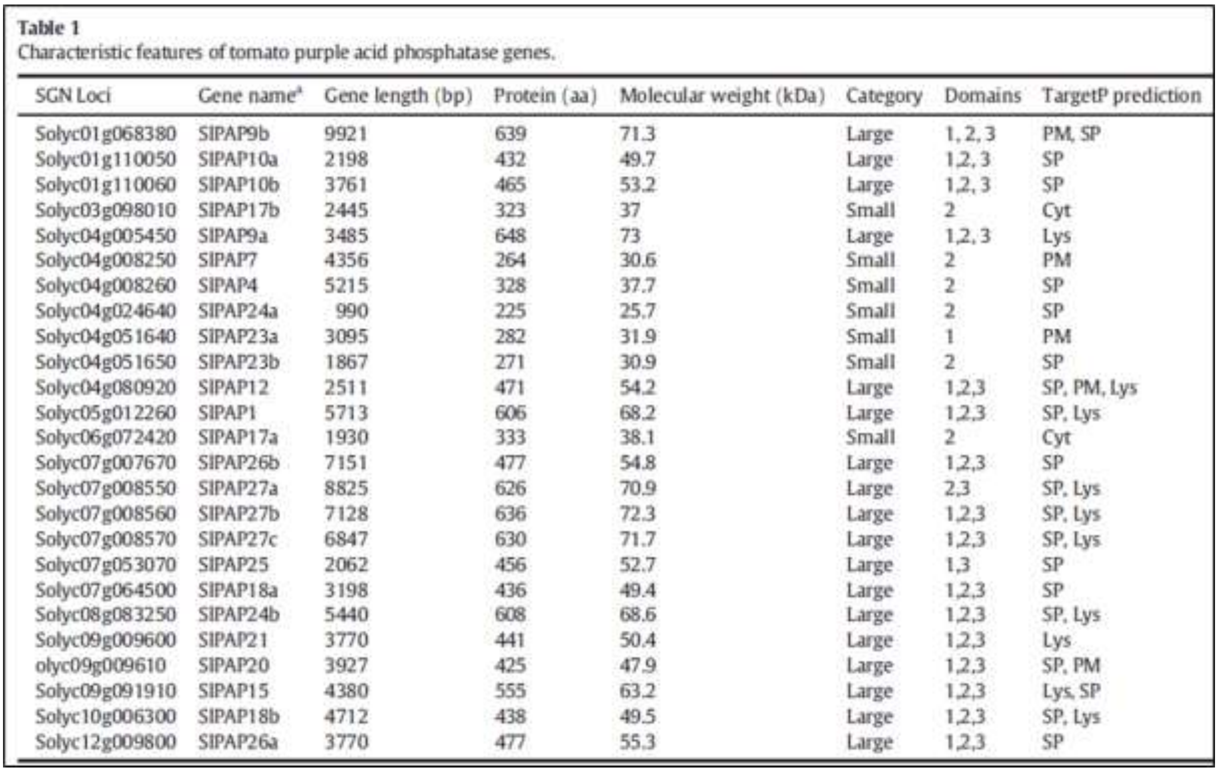


Table 1
Characteristic features of tomato purple acid phosphatase genes.

SGN Loci	Gene name[a]	Gene length (bp)	Protein (aa)	Molecular weight (kDa)	Category	Domains	TargetP prediction
Solyc01g068380	SlPAP9b	9921	639	71.3	Large	1, 2, 3	PM, SP
Solyc01g110050	SlPAP10a	2198	432	49.7	Large	1,2, 3	SP
Solyc01g110060	SlPAP10b	3761	465	53.2	Large	1,2, 3	SP
Solyc03g098010	SlPAP17b	2445	323	37	Small	2	Cyt
Solyc04g005450	SlPAP9a	3485	648	73	Large	1,2, 3	Lys
Solyc04g008250	SlPAP7	4356	264	30.6	Small	2	PM
Solyc04g008260	SlPAP4	5215	328	37.7	Small	2	SP
Solyc04g024640	SlPAP24a	990	225	25.7	Small	2	SP
Solyc04g051640	SlPAP23a	3095	282	31.9	Small	1	PM
Solyc04g051650	SlPAP23b	1867	271	30.9	Small	2	SP
Solyc04g080920	SlPAP12	2511	471	54.2	Large	1,2,3	SP, PM, Lys
Solyc05g012260	SlPAP1	5713	606	68.2	Large	1,2,3	SP, Lys
Solyc06g072420	SlPAP17a	1930	333	38.1	Small	2	Cyt
Solyc07g007670	SlPAP26b	7151	477	54.8	Large	1,2,3	SP
Solyc07g008550	SlPAP27a	8825	626	70.9	Large	2,3	SP, Lys
Solyc07g008560	SlPAP27b	7128	636	72.3	Large	1,2,3	SP, Lys
Solyc07g008570	SlPAP27c	6847	630	71.7	Large	1,2,3	SP, Lys
Solyc07g053070	SlPAP25	2062	456	52.7	Large	1,3	SP
Solyc07g064500	SlPAP18a	3198	436	49.4	Large	1,2,3	SP
Solyc08g083250	SlPAP24b	5440	608	68.6	Large	1,2,3	SP, Lys
Solyc09g009600	SlPAP21	3770	441	50.4	Large	1,2,3	Lys
olyc09g009610	SlPAP20	3927	425	47.9	Large	1,2,3	SP, PM
Solyc09g091910	SlPAP15	4380	555	63.2	Large	1,2,3	Lys, SP
Solyc10g006300	SlPAP18b	4712	438	49.5	Large	1,2,3	SP, Lys
Solyc12g009800	SlPAP26a	3770	477	55.3	Large	1,2,3	SP

#Nomenclature of tomato SlPAPs was done based on their relative homology with Arabidopsis PAPs. Domains 1, Pur_ac_phosph_N; 2, Metallophos; 3 Metallophos_C; SP, Secretory pathway; PM, Plasma membrane; Cyt, cytoplasmic; Lys, lysosomal;

2.2. Phylogenetic analysis of tomato PAPs

The phylogenetic analysis grouped the tomato, *S. pennellii*, Arabidopsis, and rice PAP proteins in three major clades **(Fig. 16)**. Both tandemly and segmentally duplicated genes were found to be closely placed in the phylogenetic tree. One of the major findings of this analysis is that direct homologs of AtPAP5, AtPAPA6, AtPAP11, AtPAP14, AtPAP16, AtPAP19, and AtPAP28 are not present in tomato and *S. pennellii* genomes. Intriguingly, while AtPAP29 homolog is present in *S. pennellii* (SpePAP29), its homolog could not be identified in tomato **(Fig. 16)**. Further, more than one Arabidopsis PAP homologs were identified for several tomato and *S. pennellii* PAPs and vice versa. For example, two or more Arabidopsis homologs were identified for tomato SlPAP21 and SpePAP29. In contrast, two or more homologs of Arabidopsis PAPs, AtPAP10, AtPAP18, AtPAP23, and AtPAP26, were identified in tomato and *S. pennellii* genomes **(Fig. 16)**. In general, PAP members of Arabidopsis, tomato and S. *pennellii* were grouped closely than their rice counterparts, indicating a greater degree of evolutionary closeness amongst dicot PAPs than their monocot homologs.

2.3. Annotation of functional and ligand binding sites in SlPAPs

Prediction of ligand binding sites for all tomato SlPAP proteins was carried out using the RaptorX binding prediction server (http://raptorx.uchicago.edu/BindingSite). Based on parameters such as P-value, unnormalized Global Distance Test (GDT), GDT, uSeqID and SeqID, RaptorX server predicts the 3-D structure model of the query amino acid sequences. This analysis discovered that the binding sites for ligands like Iron (Fe), N-acetyl-L-D-Glucosamine (NAG), Cobalt (Co), and Zinc (ZN) are conserved in most of the SlPAP proteins. Besides Fe and Zn, Mn is also predicted as a ligand for SlPAP7. Pocket Multiplicity analysis, which calculates the frequency of the selected pocket (active site) in a set of ligand-binding protein structure, predicted varied numbers (between 1 to 5) of the anticipated pockets in SlPAP proteins. Whereas SlPAP17a, SlPAP17b, and SlPAP24a were predicted to harbour one pocket each, SlPAP4 and SlPAP23b are projected to have two ligand binding sites. Zn appears to be the most preferred ligand for tomato SlPAP members with 1 or 2-pocket multiplicity. The remaining tomato SlPAP proteins have 3-5 pocket multiplicity comprising 1 to 9 binding residues. Overall, Fe and NAG were predicted to be the top ligands. The details of annotated SlPAPs for ligand binding sites are shown in **(Annexure 3)**.

2.4. SlPAP homologs exhibit diverse transcript profiles during tomato development

Scrutiny of their spatiotemporal expression in different tissues (root, leaf, stem, flower bud, and flower) and at the different stages of fruit development (1-cm, 2-cm, 3-cm, immature green, mature green, breaker, breaker+5, and breaker+10) at RNA levels in tomato and its wild relatives, *S. pimpinellifolium* and *S. pennellii* was done by mining the online available gene expression data [275][276].

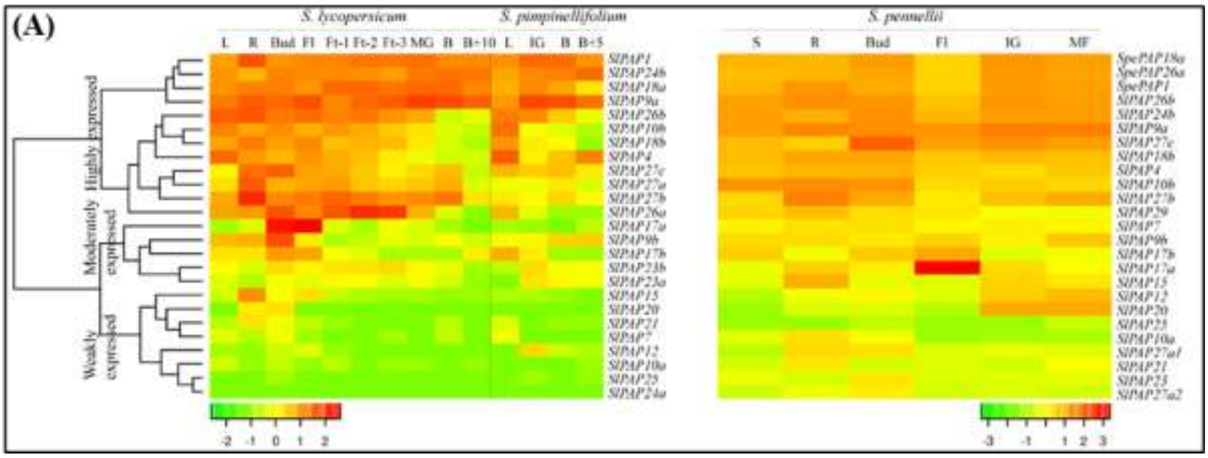


Fig 18: Expression profile of SlPAPs in tomato and its wild relatives*. *(A) *Expression profiles of purple acid phosphatases appear to be primarily conserved in tomato and its two wild*

relatives, S. pimpinellifolium and S. pennellii, during development. Expression data from the published studies (TGC, 2012; Bolger et al., 2014) were mined to retrieve profiles of SlPAP homologs. Heatmaps were generated using log2 RPKM/FPKM values in R. L, leaf; R, root; Fl, flower; Ft-1 to -3, fruit 1-cm, 2-cm, and 3-cm diameter; MG, mature green; B, breaker; IG, immature green fruit; MF, mature fruit.

This analysis divided these genes into three groups, including highly expressed (12 genes), moderately expressed (6 genes) and weakly expressed (7 genes) SlPAPs **(Fig. 18)**. We observed highly similar expression patterns of tomato PAP homologs in at least two of the three genetic backgrounds. For example, in the weakly expressed category, four members, including PAP10a, PAP20, PAP21, and PAP25 were common in all three genotypes. At least six PAP homologs, including PAP1, PAP9a, PAP15, PAP18b, PAP26b, and PAP27b, were predominantly expressed in tomato and *S. pennellii* root tissue. Further, SlPAP9a-b, SlPAP17a-b, SlPAP26a-b, and SlPAP27c homologs displayed high mRNA levels in the reproductive tissues in both tomato and *S. pennellii* **(Fig. 18)**. PAP17a homologs showed exceptionally high expression in flowers in both tomato and *S. pennellii*. Similarly, several PAP homologs, including PAP1, PAP9a, PAP18a, PAP24b and PAP26b showed higher transcript levels at mature fruit/ripening stages in all three genetic backgrounds **(Fig. 18)**.

2.5. Transcriptome study of Pi starved seedlings at 8-D and 15-D time points

Next, we studied transcriptomes of tomato seedlings (unpublished data, Akash et al.) to identify phosphorous starvation inducible (PSI) genes on a global scale. To provide a framework for understanding how tomato genes respond to Pi starvation, we compared mRNA populations from 8-D and 15-D HP-grown seedlings transcriptome with that of 8-D and 15-D LP- grown seedlings, respectively. Genes altered by at least 2-fold (log2=1) at p-value ≤0.05 in their mRNA levels in the DESeq analysis were considered differentially regulated. In our in-house transcriptome data, 21 SlPAPs were detected. Higher expression of *SlPAP7*, *SlPAP10b*, *SlPAP12*, *SlPAP15*, *SlPAP17b* and *SlPAP26b* at both time points post starvation explains their Pi responsive nature **(Fig. 19A)**. Our data on PAPs found a good correlation with earlier published transcriptome data by Pfaff et al. (2020) where these genes have also been reported to be early responsive to Pi starvation[277]. We also studied tomato seedlings' proteomes and observed a higher abundance of SlPAP10b, SlPAP15, and SlPAP26b peptides in LP-grown seedlings than their HP counterparts. Besides this, the proteome data also observed moderately higher peptide levels of other SlPAPs, such as SlPAP1, SlPAP10a, and SlPAP27b **(Fig. 19B)**.

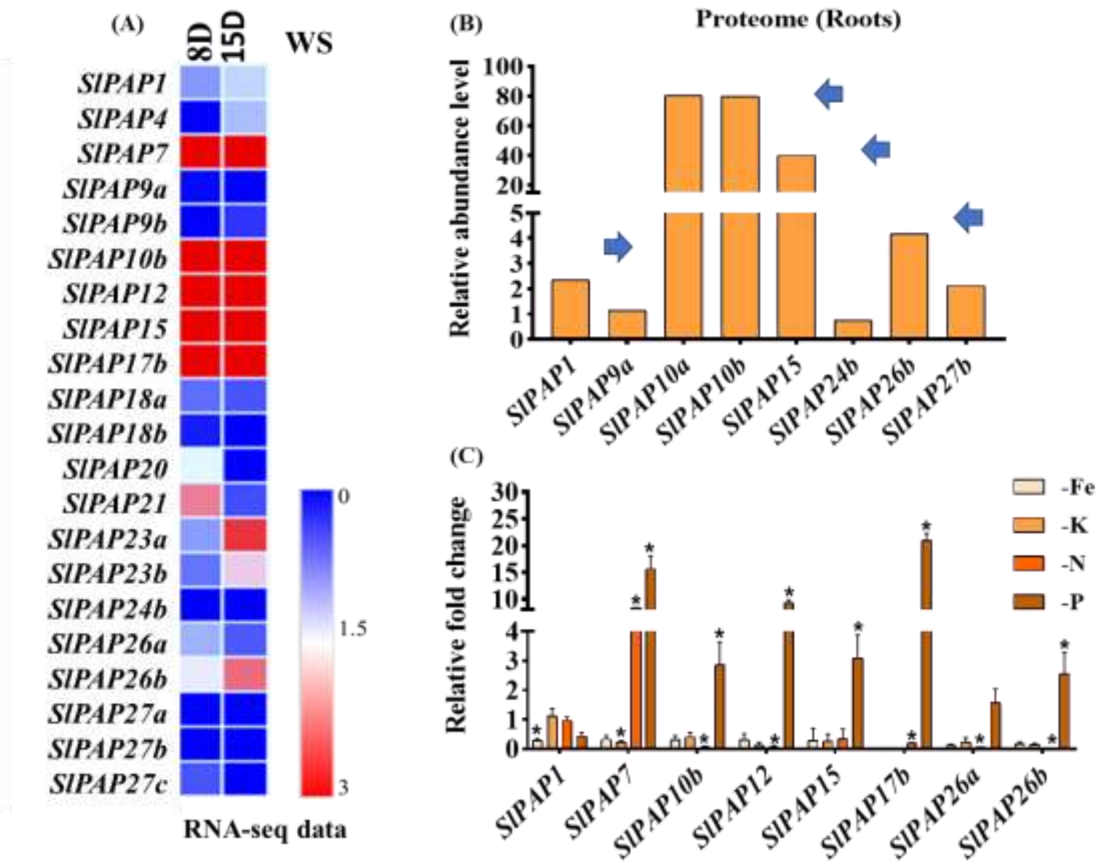


Fig 19: Expression profiling of SlPAPs* in HP and LP-grown seedlings (A)** *Identification of differentially regulated SlPAPs under Pi starvation condition at 8-D and 15-D of Pi starvation in tomato seedlings of Pusa Ruby cultivar.* ***(B) *Validation of the selected highly expressed SlPAPs at the protein level.* ***(C)*** *Expression profile of a selected candidate SlPAPs in different minerals stresses. –P= without phosphate, –Mg= without magnesium, –Ca= without calcium, –N= without nitrogen and –K= without potassium. Error bar shows standard deviation. Plants grown in Hoagland's medium with 1.25 mM Pi were used as a control to calculate the relative gene expression.*

The differentially regulated SlPAPs in transcriptome data were validated in the root and shoot tissues of HP- and LP-grown tomato seedlings using RT-qPCR at 8-D and 15-D time points. We studied the transcript abundance of all 25 SlPAPs. We found higher expression of seven genes in the root and 12 genes in shoot tissue at 8D post Pi starvation **(Fig. 20A).** At 15D, 14 SlPAPs in the root and 7 SlPAPs in the shoot tissue were upregulated **(Fig. 20B).** Based on our RT-qPCR data, *SlPAP9a*, *SlPAP18b*, *SlPAP18a* and *SlPAP27b* were found to be shoot-specific on both time points. At the same time, *SlPAP4*, *SlPAP15*, *SlPAP25*, and *SlPAP27a* are root-specific. This analysis showed that 20 SlPAP genes are induced, although to varied levels, at least at one of the time points under Pi deficiency **(Fig. 20)**.

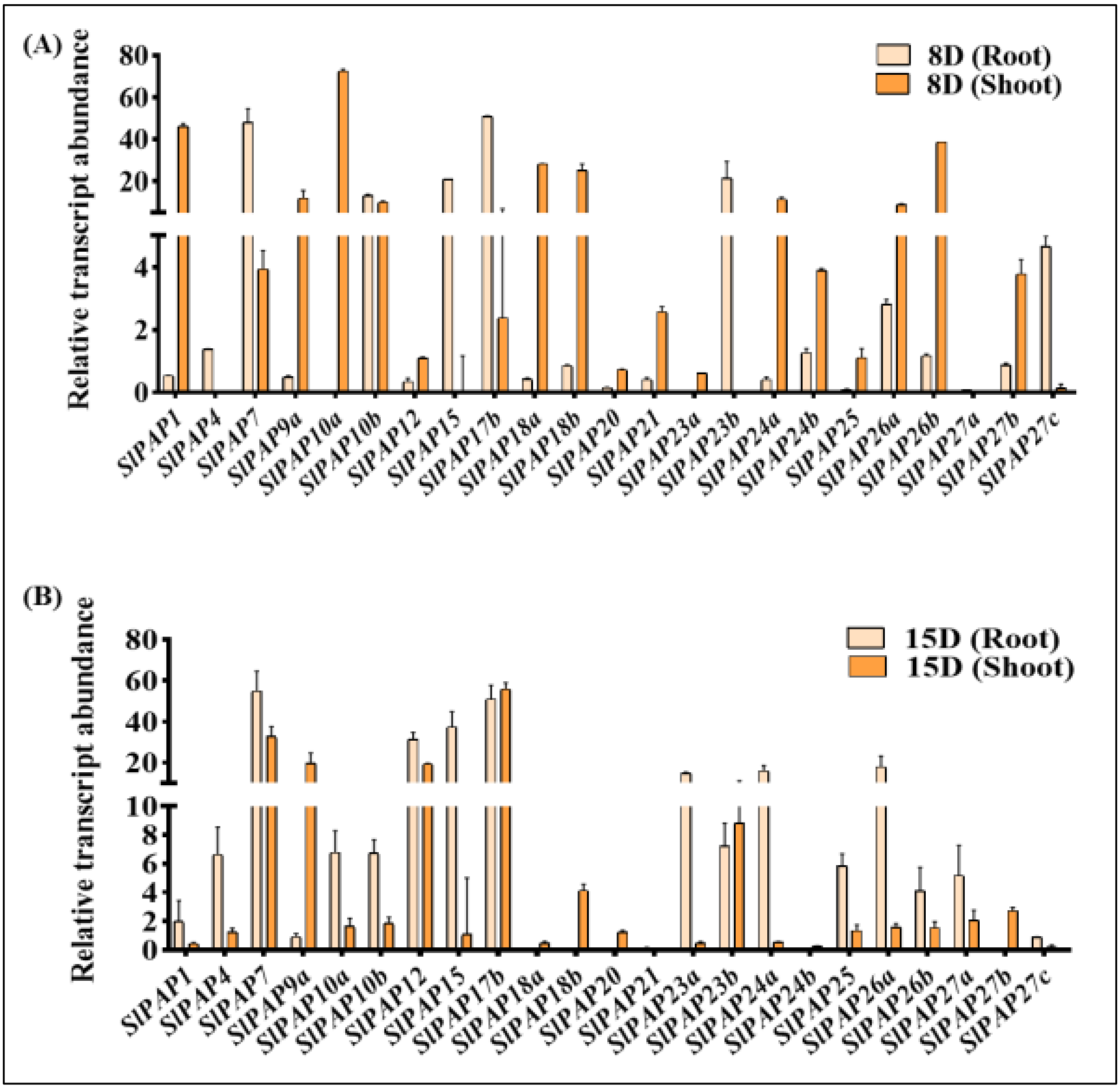


Fig 20: Validation of RNA-seq results using RT-qPCR approach. *Relative expression profiles of SlPAPs in root- and shoot-tissues under Pi deficiency (5 µM) after 8-day* ***(A)*** *and 15-day (****B****). Plants grown in Hoagland's medium with 1.25 mM Pi were used as a control to calculate the relative gene expression. SlGAPDH was used as an endogenous control.*

2.6. Influence of Pi nutrition on mRNA abundance of SlPAPs

Among the PSI SlPAP genes, *SlPAP1*, *SlPAP7*, *SlPAP10a*, *SlPAP12b*, *SlPAP15*, *SlPAP17b*, and *SlPAP26b* showed the highest induction under Pi starvation. Upon further investigations, it was noticed that *SlPAP1* and *SlPAP26b* are highly induced in shoots at both time points, whereas *SlPAP7*, *SlPAP15*, and *SlPAP17b* are induced at higher levels only in roots **(Fig. 20A-B)**. A probe of P1BS element, an element which serves as a binding site for PHR transcription factors, in the promoters of tomato SlPAPs showed that one to five P1BS elements are present in the 3-kb upstream promoter regions of PSI SlPAP genes, except SlPAP21, SlPAP23a, and

SlPAP25, which lacked this cis-element **(Fig. 15D)**. However, apart from having higher expression upon Pi starvation, we also wanted to know whether SlPAPs are induced under other mineral nutrient deficiencies. For this, we also checked the mRNA abundance of highly induced PSI PAPs, including, *SlPAP1*, *SlPAP7*, *SlPAP10b*, *SlPAP12*, *SlPAP15*, *SlPAP17b*, *SlPAP26a* and *SlPAP26b* under the deficiency of N, K, and Fe **(Fig. 19C)**. This analysis confirmed the PSI nature of *SlPAPs* as the transcript accumulation of most selected genes.

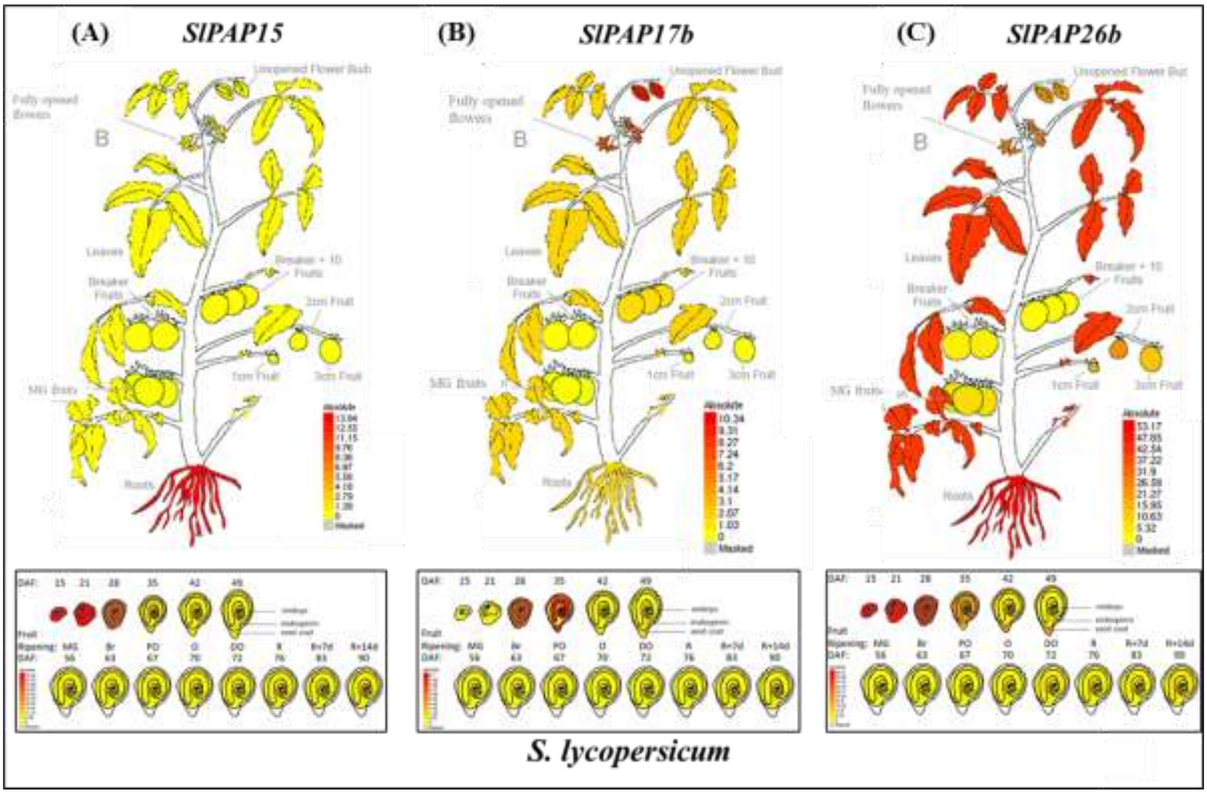


Fig 21: Expression of candidate SlPAPs at different stages of plant development (A-C) *Expression analysis of SlPAP15, SlPAP17b, and SlPAP26b gene in different stages/tissues/organs of tomato development. The figures are developed using Multi-Plant eFP Browser 2.0 – BAR tool. http://bar.utoronto.ca/efp2/Tomato/Tomato_eFPBrowser2.html*

Based on their Pi-specific nature and varied expression profiles in different tissues/organs and at different plant growth and development stages, we selected PSI genes *SlPAP15*, *SlPAP17b*, and *SlPAP26b* for detailed characterization **(Fig. 21)**. The expression of *SlPAP15* is root preferential in nature, while that of *SlPAP17b* is more in flower buds and seeds. *SlPAP26* in tomato has two close paralogs, *SlPAP26a* and *SlPAP26b* **(Fig. 21A-C)**. Due to the ubiquitously low expression of SlPAP26a in tomato tissues, this gene was not further selected for its detailed characterization. In contrast, the transcripts level of *SlPAP26b* is ubiquitous at various stages of tomato growth and development. Cloning of gene fragments for VIGS for all three genes

SlPAP15, *SlPAP17b,* and *SlPAP26b* was done in the *EV* **(Fig. 22A, B).** Double digestion confirmed the cloning of these fragments **(Fig. 18E)**. *Phytoene desaturase* (*SlPDS*) was used as a positive control of the VIGS experiments, as its silencing led to a photobleached phenotype of leaves in plants. Based on the total number of photobleached seedlings in *SlPDS*-silenced plants, the efficiency of VIGS experiment was found to be around 90 % **(Fig. 23A-D)**.

Experimental design to study phosphate starvation responses in tomato seedlings

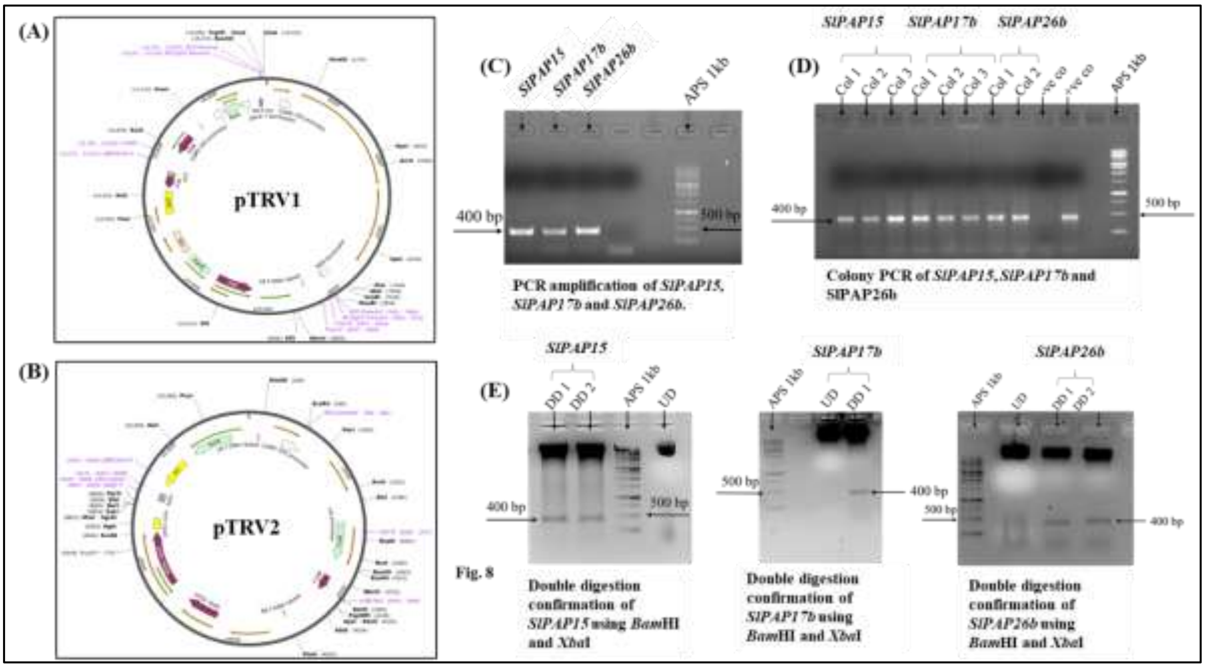


***Fig 22: VIGS Cloning of selected SlPAPs in pTRV2 vector (A-B)** Vector maps of pTRV1 and pTRV2 VIGS plasmids. The pTRV2 was used for cloning of SlPAP15, SlPAP17b, and SlPAP26b VIGS fragments.* ***(C)** PCR amplification of 400 bp VIGS fragments.* ***(D-E)** Colony PCR and restriction double digestion confirmation of SlPAP15, SlPAP17b and SlPAP26b VIGS plasmids.*

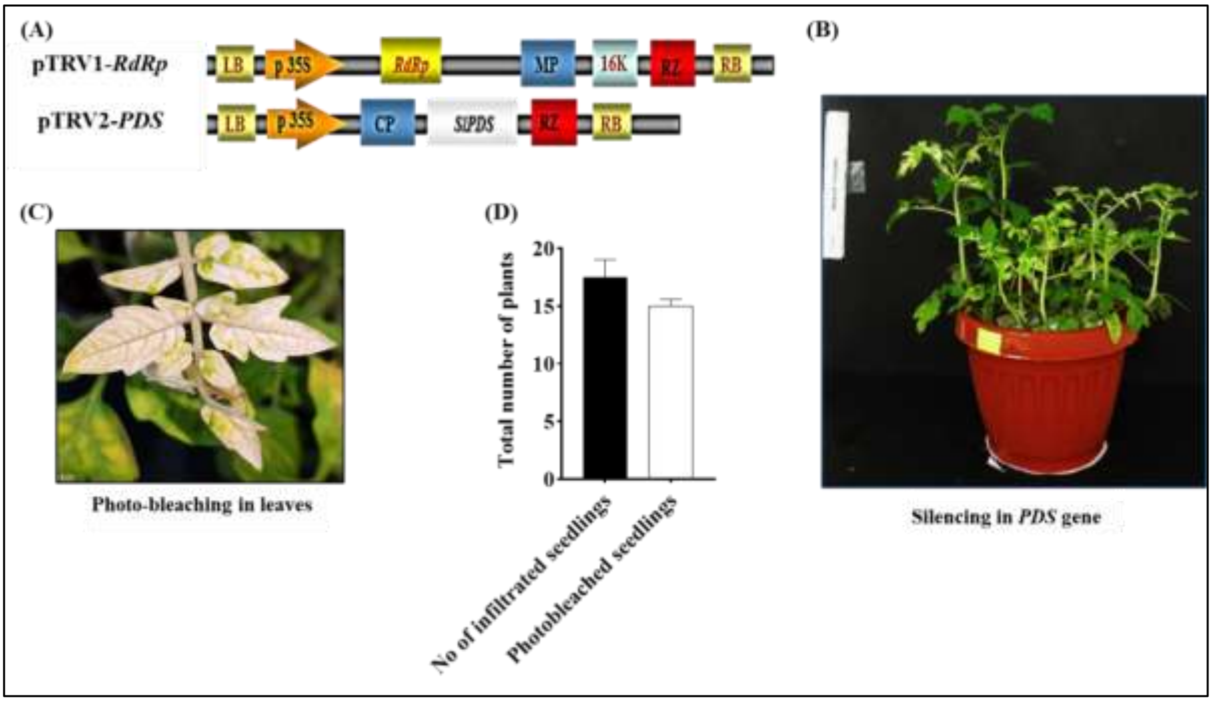


Fig 23: Efficiency of VIGS was measured using SlPDS as a positive control. (A) *SlPDS and SlPAPs VIGS fragments were cloned between CaMV35S promoter (2X35S) and nopaline synthase terminator (NOSt) in pTRV1. pTRV2-SlPDS is a representative vector diagram for SlPAPs VIGS constructs. RdRp, RNA dependent RNA polymerase; 16 kDa cysteine-rich protein; MP, movement protein; CP, coat protein; LB and RB, left and right borders of T-DNA; Rz, self-cleaving ribozyme; MCS, multiple cloning sites.* ***(B-C)*** *Silencing of SlPDS resulted in photobleached leaves in the tomato seedlings.* ***(D)*** *The efficiency of VIGS experiments was calculated by counting the total number of photobleached plants in SlPDS-infiltrated plants.*

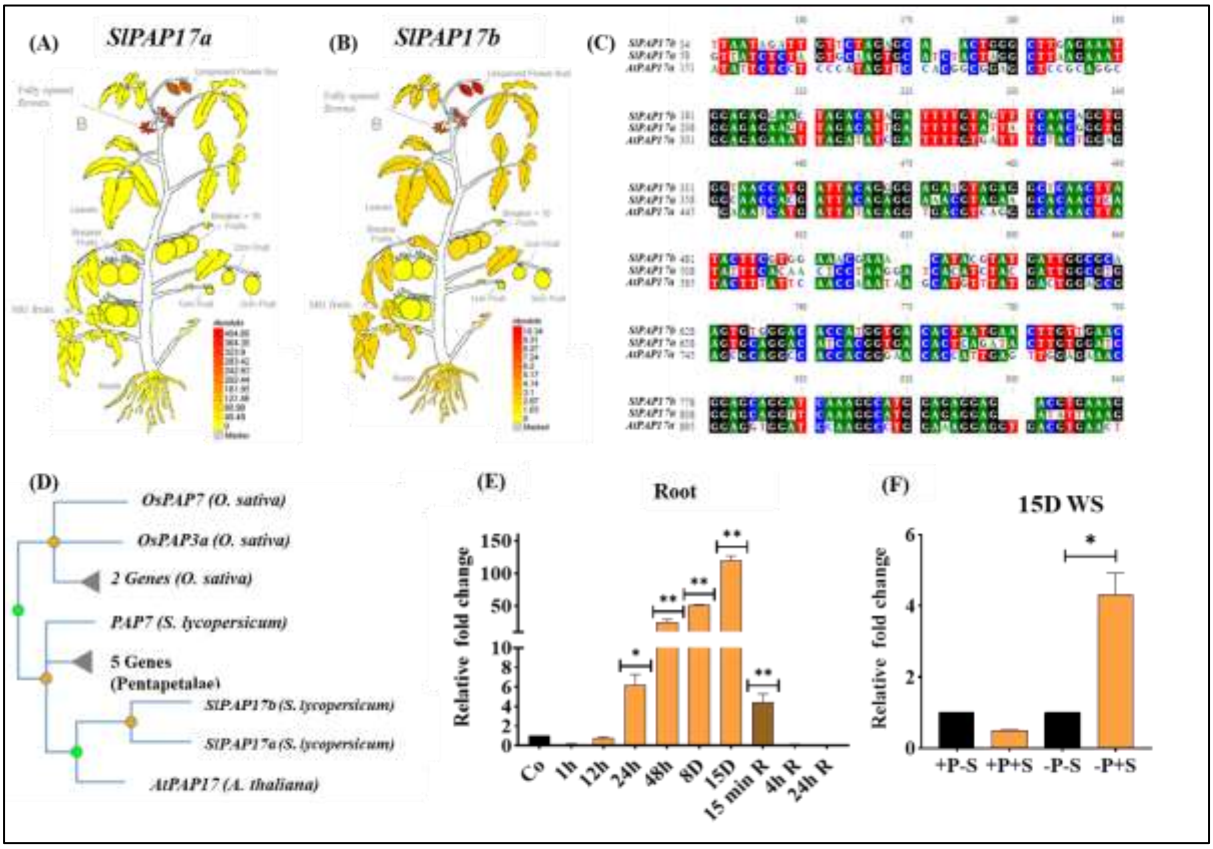


Fig 24: Expression analysis and Insilico study of SlPAP17 (A-B) *Expression analysis of SlPAP17a and SlPAP17b gene at different development stages using multi-Plant eFP Browser 2.0 – BAR tool.* ***(C)*** *Multiple sequence alignment of SlPAP17 paralogs in tomato with the Arabidopsis homologs was performed using CLUSTALX and BioEdit.* ***(D)*** *Phylogeny of SlPAP17 paralogs was created using PhyloGenes tool on the SGN website.* ***(E)*** *Time course kinetics and recovery of SlPAP17b upon Pi starvation and resupply.* ***(F)*** *Expression profiling of SlPAP17b upon low Pi with and without the exogenous sucrose supply. +P-S = (HP without sucrose), +P+S (HP with sucrose), -P-S (LP without sucrose), -P+S = (LP with sucrose).*

2.7. *SlPAP17b* is an early Pi starvation-responsive gene.

SlPAP17 has two paralogs, *SlPAP17a,* and *SlPAP17b*. Both genes are the close homolog of Arabidopsis PAP17 **(Fig. 24A, B)**. Characterization of AtPAP17 revealed its dual localization to the extracellular matrix and lytic vacuoles. This gene is upregulated at the low Pi condition and involved in leaf senescence and oxidative stress **(Fig. 24C, D)**. The percentage similarity of *SlPAP17a*/b at the nucleotide level with AtPAP17 is ~74 % **(Fig. 24C)**. Due to the non-PSI nature and its weak expression in tomato plants, we excluded *SlPAP17a* from further experiments. *SlPAP17b* is highly induced at 8-D and 15-D of Pi starvation. Time-course experiment studying the transcripts of *SlPAP17b* at different time points showed its induction after 24h post starvation, indicating it to be an early PSI gene. An immediate recovery in its transcripts after P resupply implies SlPAP17b to be highly Pi-dependent **(Fig. 24E)**. Higher induction of *SlPAP17b* under LP condition only in the presence of exogenously supplied sucrose shows that its transcripts level is positively influenced by this sugar **(Fig. 24F)**.

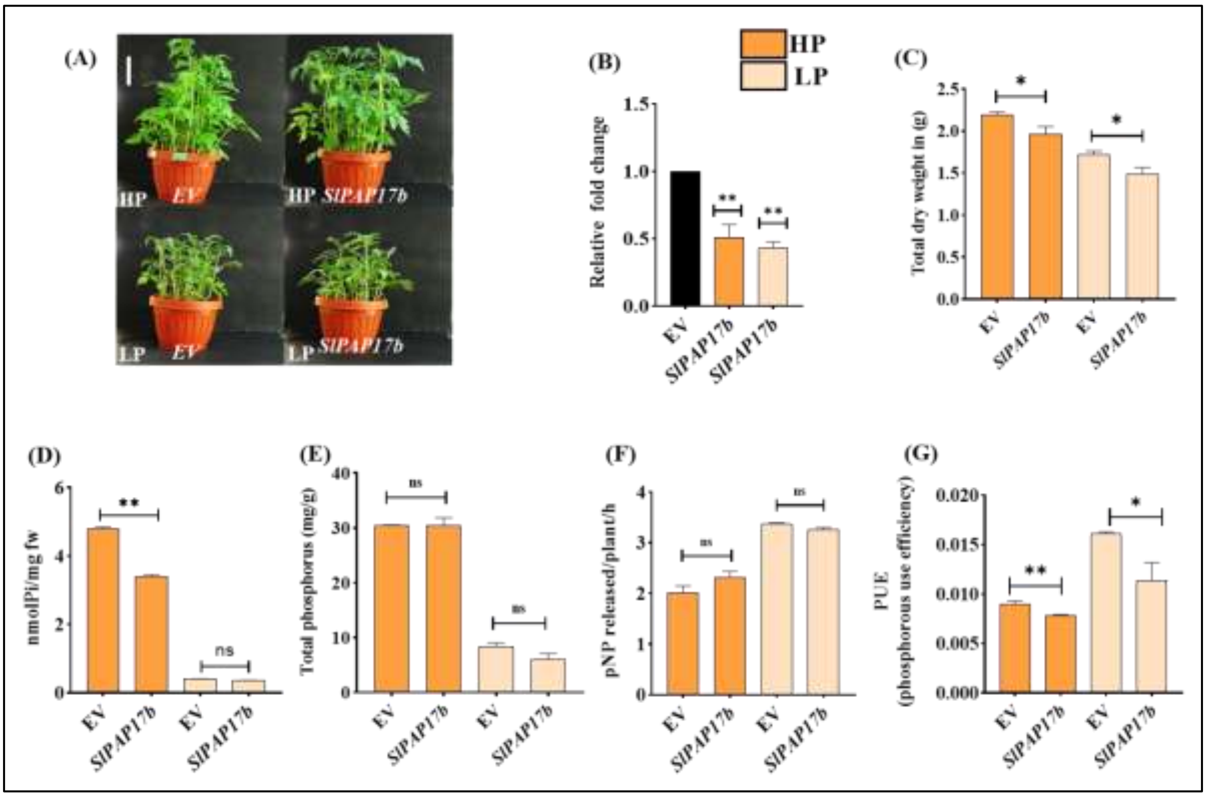


Fig 25: Characterization of SlPAP17b using VIGS (A) *Seedlings phenotype of silenced plants* ***(B)*** *Confirmation of silencing of SlPAP17b using RT-qPCR* ***(C)*** *Total dry weight was measured in gram in silenced and unsilenced seedlings* ***(D)*** *Total soluble Pi content of the SlPAP17b silenced seedlings in HP and LP conditions* ***(E)*** *The total P content of the SlPAP17b silenced seedlings in HP and LP conditions* ***(F)*** *Secretory APase activity in the SlPAP17b silenced seedlings* ***(G)*** *PUE (Phosphorus use efficiency) in the silenced and unsilenced seedlings.*

2.7.1. Functional characterization of *SlPAP17b* using VIGS

To get primary insight into the function of *SlPAP17b* during PSR, we decided to silence this gene using VIGS transiently. Using standard and lab-optimized VIGS protocols by Senthil Kumar et al. (2013) and Akash et al. (2022), we used *pTRV2:SlPAP17b* construct to induce silencing of this gene in tomato plants[268,278]. The silencing of *SlPAP17b* was confirmed by RT-qPCR analysis using gene-specific primers **(Fig. 25B)**. As a positive control for the VIGS experiments, we also silenced *Phytoene desaturase* (PDS) genes and scored the characteristic bleaching leaf phenotype in the transiently silenced tomato seedlings. Overall, excellent silencing efficiency of *SlPDS* (over 80 %) was observed in the repeated VIGS experiments **(Fig. 23A-D)**. The silencing of this gene was found to modulate plant response to Pi starvation at morpho-physiological, biochemical, and molecular levels indicating perturbed PSR in the silenced tomato seedlings.

2.7.2. Silencing of *SlPAP17b* leads to altered Pi content in both Pi-sufficient and Pi-deficient seedlings

We first investigated the repercussion of *SlPAP17b* VIGS at the phenotypic level in both HP and LP conditions **(Fig. 25A)**. We did not observe any significant change at the phenotypic level. However, the plant's dry weight has reduced in both HP and LP conditions. We also found that silenced seedlings accumulated lower levels of total soluble Pi under HP condition specifically, indicating the perturbed Pi remobilization **(Fig. 25C)**. No difference was observed in the total P content of silenced and empty vector (EV) control seedlings **(Fig. 25D)**. PUE in silenced seedlings reduced significantly compared to EV controls **(Fig. 25F)**, indicating it to be one of the important SlPAPs controlling Pi recycling inside the plant. We also checked secretory APase activity in the silenced and EV control seedlings but could not detect any significant difference between the two sets of plants under HP or LP conditions **(Fig. 25E)**.

2.8. Functional characterization of *SlPAP26a* and *SlPAP26b,* cytosolic proteins with differential expression levels at different stages of plant development

SlPAP26b is one of the closest homologs of Arabidopsis *AtPAP26* in tomato **(Fig. 26A)**. Multiple sequence alignments of *SlPAP26a* and *SlPAP26b* with *AtPAP26* have shown 81 % and 83 % sequence similarity at the nucleotide level **(Fig. 26B)**. In the RNA-seq data, *SlPAP26b* was found to have higher expression in root, shoot, leaves, and to some extent, at the initial stage of fruit development **(Fig. 26D)**. RT-qPCR further validated this observation, which confirmed the highest mRNA abundance of this gene in root, leaf and flower tissues **(Fig. 26E)**. In contrast, its paralog *SlPAP26a* is weakly expressed throughout all plant growth and development stages **(Fig. 26C)**. Functional characterization of *SlPAP26a* also confirmed its negligible role in controlling phosphate starvation responses. Silencing of this gene resulted in no change at the phenotypic and biochemical level in tomato seedlings. Upon silencing, no major difference was observed in total soluble Pi content, SAP, and total anthocyanin contents in the silenced and EV seedlings in both HP and LP conditions **(Fig. 26A-F)**. As we did not notice the influence of silencing of *SlPAP26a*, we took *SlPAP26b* for its functional characterization.

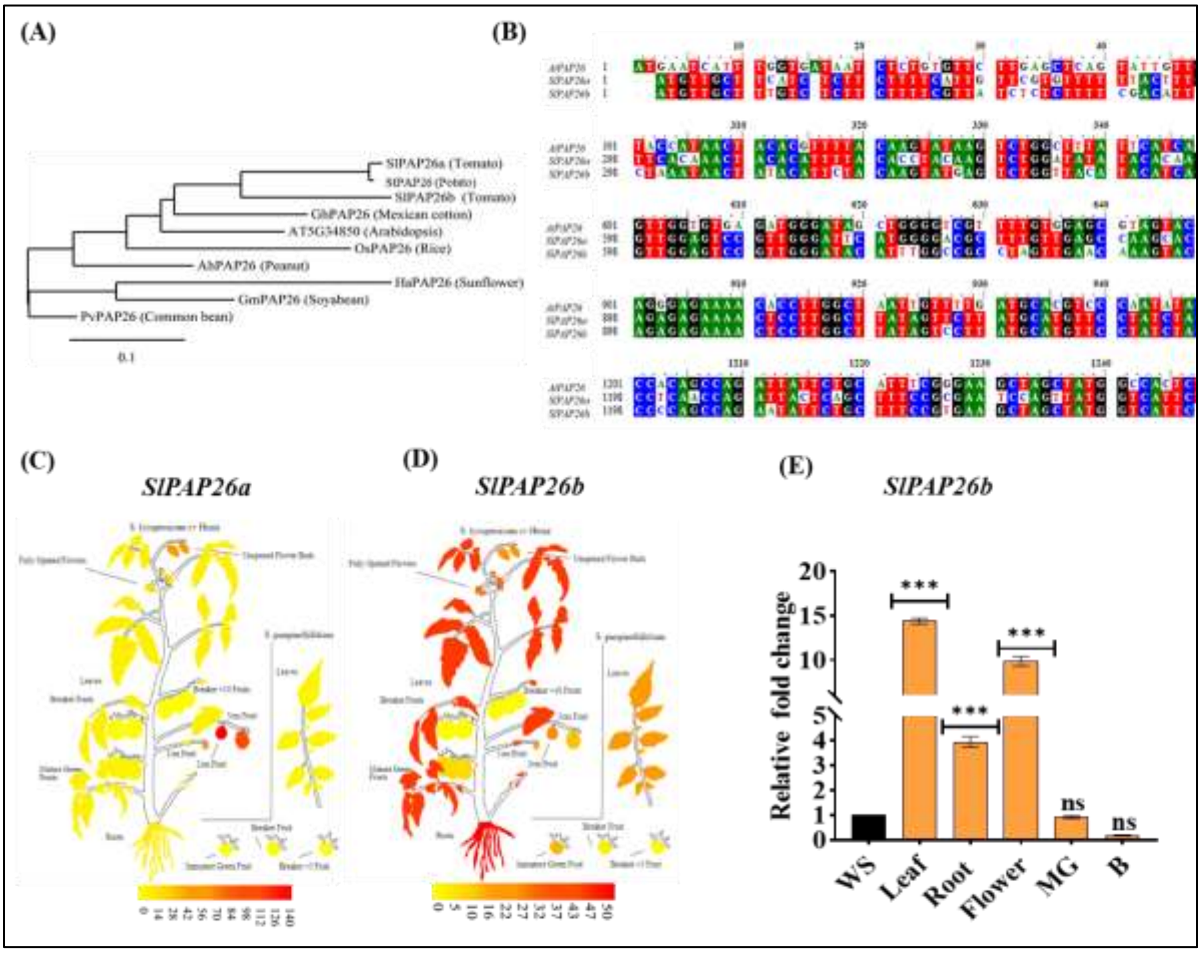


Fig 26: Expression analysis and Insilico study of SlPAP26 (A) *Phylogeny of SlPAP26 genes was constructed using PhyloGenes in the SGN tool.* ***(B)*** *Multiple sequence alignment of SlPAP26 paralogs and AtPAP26 was constructed using CLUSTALW and BioEdit.* ***(C-D)*** *Expression analysis of SlPAP26a and SlPAP26b gene at different development stages using multi-plant eFP Browser 2.0 – BAR tool.* ***(E)*** *Validation of SlPAP26b transcript levels using RT-qPCR. WS; whole seedlings, MG; mature green, B; breaker.*

2.8.1. *SlPAP26b* is an early Pi starvation-responsive gene

We also perform time-course kinetics and recovery experiments of *SlPAP26b* using RT-qPCR. Its induction within 24 hr of Pi starvation established this as an early PSI gene. Pi resupply lowered its expression to the levels observed in Pi-sufficient seedlings, indicating its tight regulation by intracellular Pi levels. Its induction is rapid and more pronounced in the shoot tissue than in the root **(Fig. 27A, B)**. An exogenous supply of sucrose led to an enhanced transcripts level of this gene, making it a suitable target gene for its functional characterization **(Fig. 27C)**.

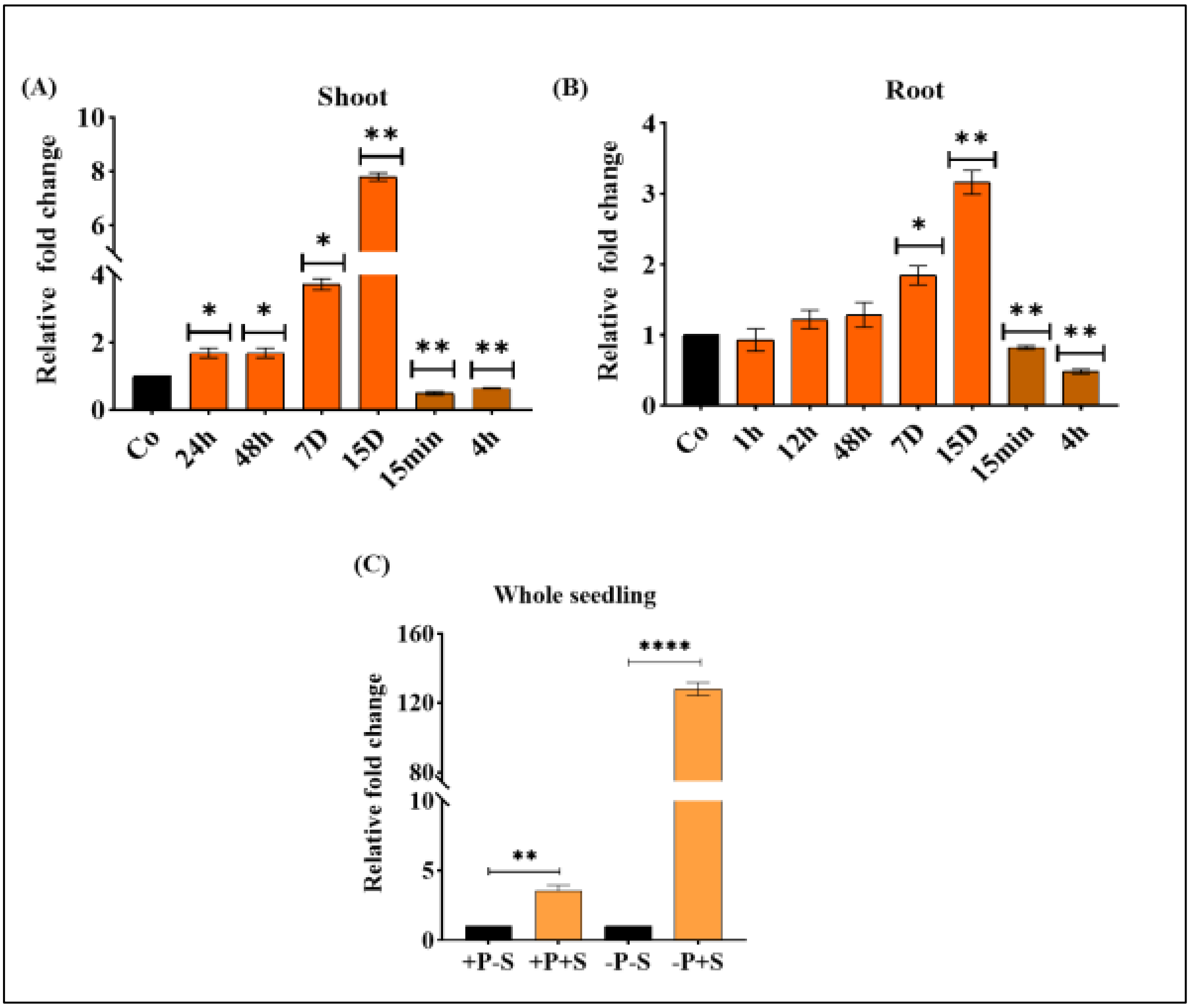


Fig 27: Transcript profiling of SlPAP26b in a time-course study and its induction upon sucrose supplementation (A) *Time course kinetics and recovery of SlPAP26b in Pi-starved shoot and root tissues.* ***(B-C)*** *The expression of SlPAP26b with and without exogenous sucrose supply under HP and LP conditions.*

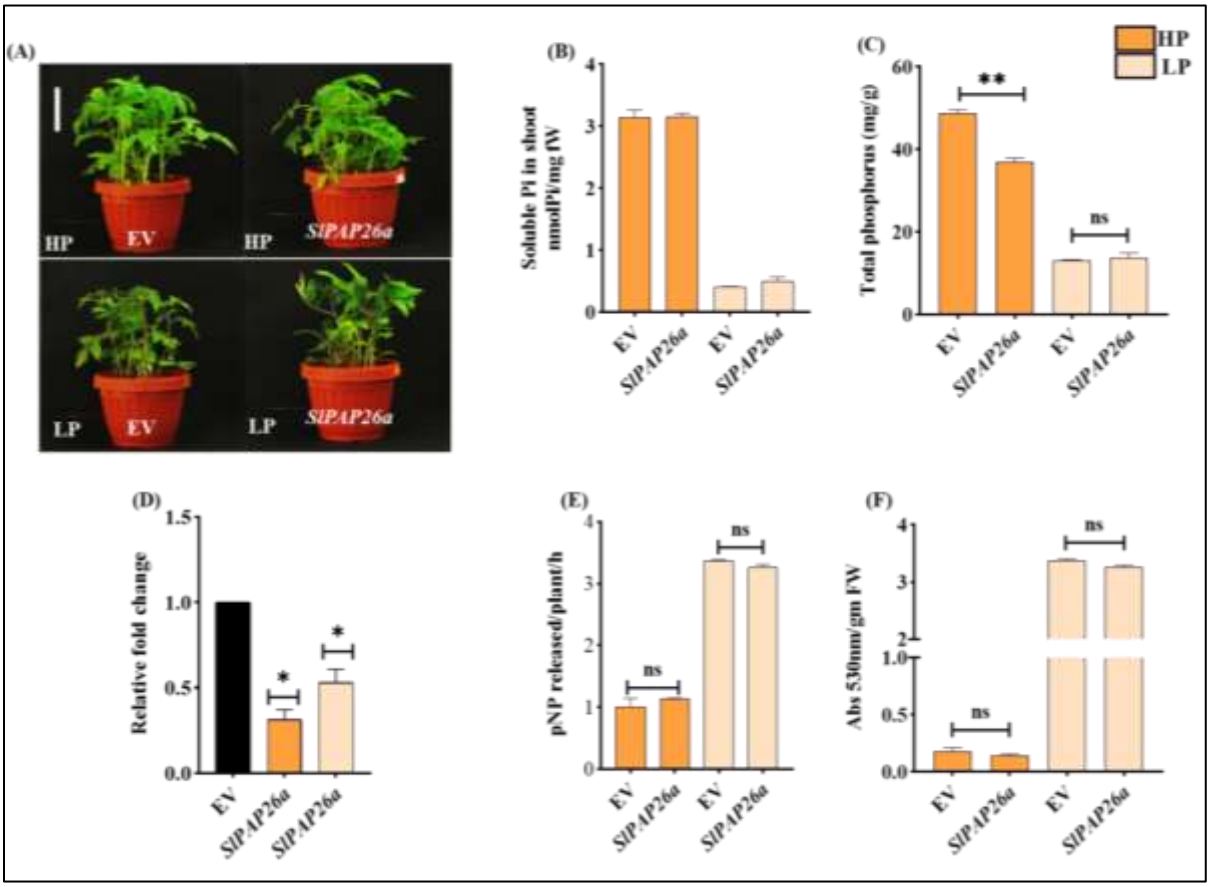


Fig 28: Silencing of SlPAP26a using VIGS (A) *Seedlings phenotype of EV and SlPAP26a silenced plants under HP and LP conditions* ***(B)*** *Total soluble Pi content in shoot.* ***(C)*** *Total P content in seedlings* ***(D)*** *Confirmation of SlPAP26a silencing at transcripts level using RT-qPCR* ***(E)*** *Secretory acid phosphatase activity using pNPP as substrate.* ***(F)*** *Total anthocyanins content in silenced and unsilenced seedlings under HP and LP conditions.*

2.8.2. Silencing of *SlPAP26b* perturbs Pi homeostasis in the silenced plants

Next, *SlPAP26b* was transiently silenced using VIGS, and the infiltrated plants were confirmed as described previously for *SlPAP17b*. The *SlPDS* gene was used as a positive control in the VIGS experiments. Photobleaching of leaves was observed in all *SlPDS*-silenced plants, indicating an excellent gene silencing efficiency. The infection of EV controls was confirmed by PCR analysis using coat protein–specific gene primers. The RT-qPCR analysis confirmed the silencing of *SlPAP26b* gene in both Pi-sufficient and Pi-deficient plants compared to the EV controls **(Fig. 29B)**. *SlPAP26b* transcripts were downregulated by 65 % in the Pi-sufficient conditions, and 60 % inhibition in its transcripts was observed in Pi-deficient conditions in the silenced plants compared to EV seedlings**.**

2.8.3. *SlPAP26b* silenced plants accumulated lower Pi levels and exhibited subdued secretory acid phosphatase activity

SlPAP26b silencing resulted in stunted plant growth and appeared more sensitive under LP conditions **(Fig. 29A)**. Higher accumulation of total anthocyanins content and increased primary root length in silenced seedlings under LP conditions confirmed these plants' exacerbated Pi stress response **(Fig. 29D)**. It also resulted in a lower accumulation of soluble Pi in silenced plants than EV controls under HP and LP conditions **(Fig. 29E)**. The silenced plants also accumulated lower total phosphorus levels than their EV control **(Fig. 29F)**. These plants also showed significantly lower SAP activity, mainly under LP conditions, than their controls **(Fig. 29G)**. This soluble Pi and decreased SAP activity were further confirmed at molecular levels by RT-qPCR of selected PSI phosphate transporter1 (SlPT1) and acid phosphatase (SlPAP) genes. *SlPT1* and *SlPT7* transcripts increased in the silenced plants, especially under LP conditions, indicating the severity of PSR in the silenced plants.

2.8.4. Impact of *SlPAP26b* silencing on endogenous carbohydrate content and acid phosphatase activity in plants.

Acid phosphatase plays a crucial role in maintaining the plant Pi status by breaking sugar-conjugated phosphate, such as glucose-6-phosphate, fructose-6-phosphate etc. Higher sucrose, D-glucose, and D-fructose content in root were noticed in silenced seedlings under LP conditions, while the same is observed under HP conditions except for D-glucose. In the shoot tissue, higher carbohydrate content was observed under LP conditions. Higher D-glucose content was noticed among the three sugars in the Pi-sufficient seedlings **(Fig. 30A, B)**. In-gel assay for estimating internal acid phosphatase activity showed lower activity of SlPAP26b (54.8 kDa) in both root and shoot tissues of the silenced plants under HP and LP conditions **(Fig. 30C-D).**

2.8.5. Expression of PSI genes is affected upon *SlSPX26b* silencing

Higher expression of *SlPAP15*, *SlPAP17b*, *SlPAP26a*, *SlPT1* and *SlPT7* was noticed in silenced lines under LP condition, majorly in root (FC ≥2-fold) than in shoot where induction was observed in *SlPAP26a* only **(Fig. 31A, B)**. As the regulation of PSI genes is under the control of SPX-PHR module, we also checked the expression of SlSPX2 at the protein level using western blotting **(Fig. 31C, D)**. Higher accumulation of SlSPX2 was noticed in both the EV control of shoot and root under HP condition than in LP condition. While in silenced plants, no change in SlSPX2 levels was observed in the shoot under HP and LP conditions.

However, the SlSPX2 level has decreased in silenced lines under LP condition in root, but no change was observed under HP condition **(Fig. 31C, D)**.

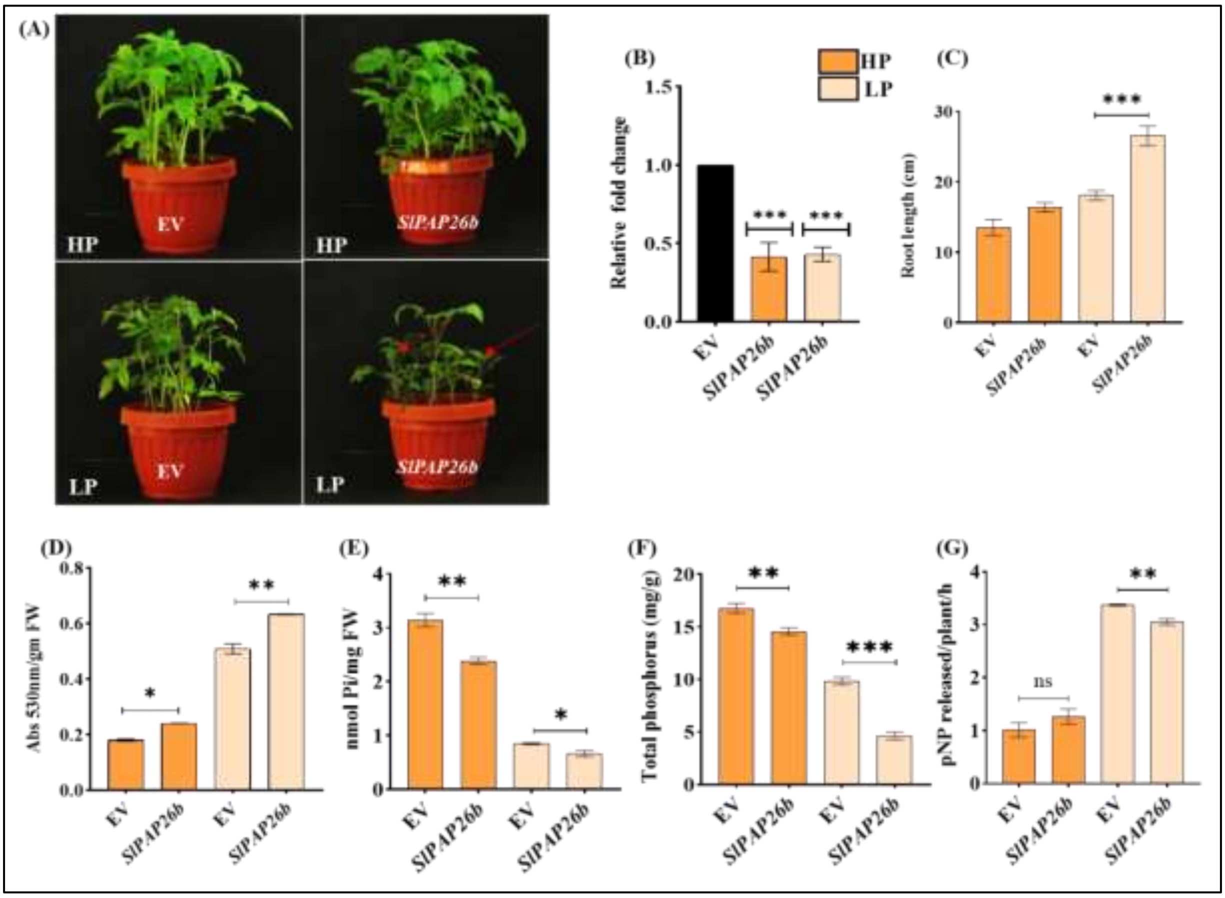


Fig 29: Silencing of SlPAP26b using VIGS (A) *Seedlings phenotype of EV controls and SlPAP26b silenced plants under HP and LP conditions **(B)** Confirmation of SlPAP26b silencing at transcripts level using RT-qPCR **(C-D)** Root length and total anthocyanins content. **(E)** Total soluble Pi content **(F)** Total P content **(G)** Root-associated total secretory acid phosphatase activity (SAP) in EV and SlPAP26b-silenced seedlings.*

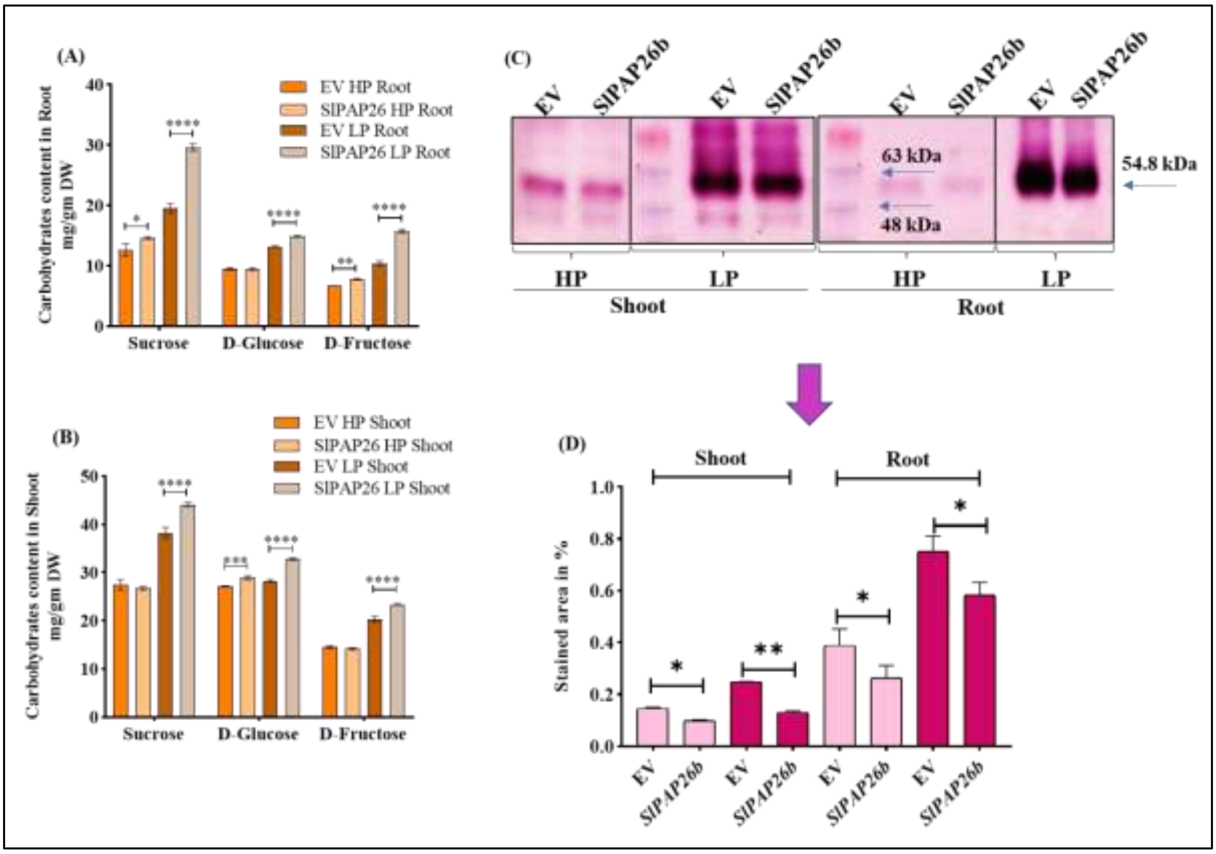


Fig 30: Change in total carbohydrate content and APase activity in silenced and unsilenced seedlings under HP and LP condition (A) *Estimation of monosaccharide and disaccharide sugars in silenced and EV seedlings* ***(B)*** *Level of D-glucose, D-fructose, and sucrose was measured in root and shoot tissues.* ***(C)*** *In-gel assay in SlPAP26b silenced and EV plants were performed in root and shoot tissues separately****.*** ***(D)*** *Quantification of APase activity detected in-gel using ImageJ software.*

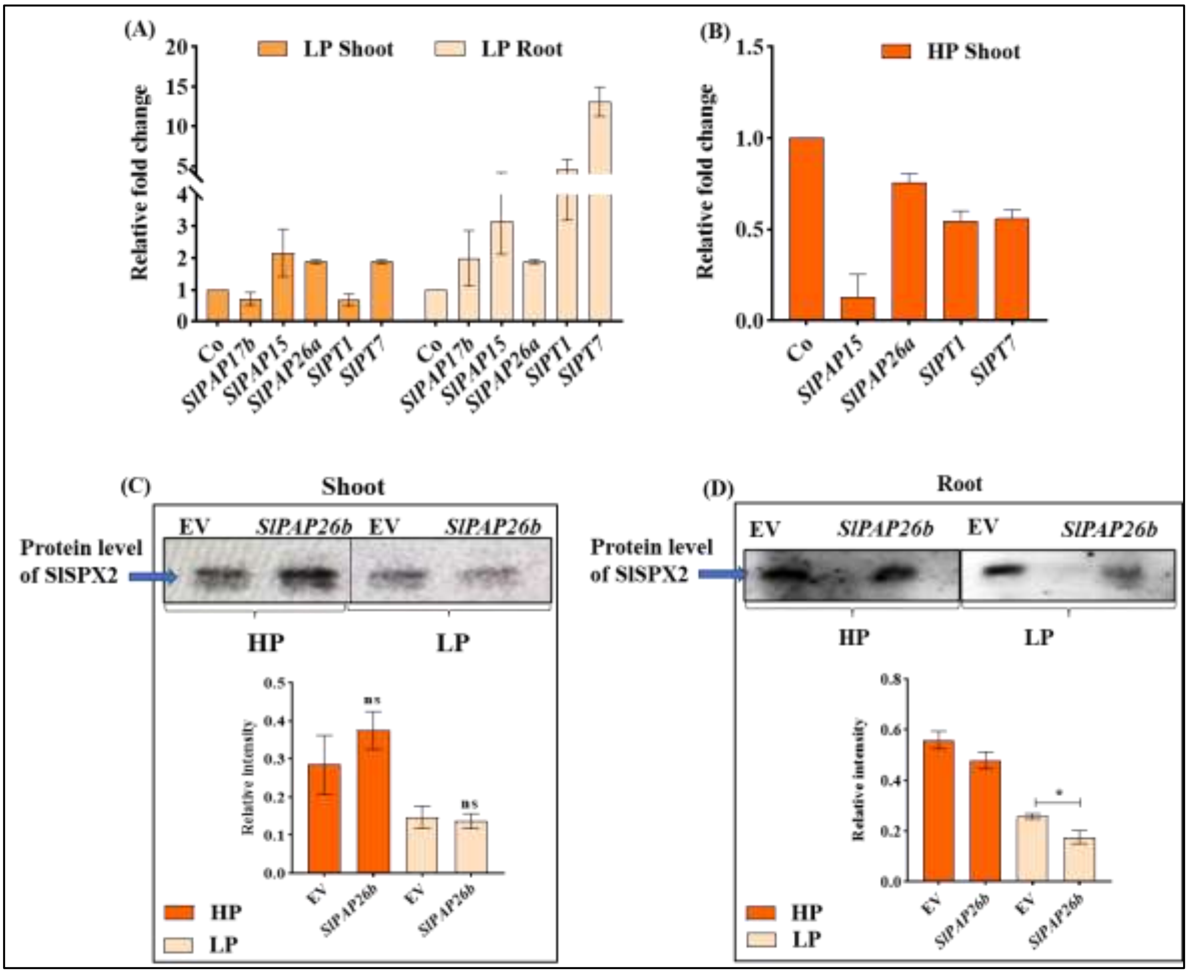


Fig 31: ***Expression profile of PSI genes in silenced lines. (A-B)*** *Expression of candidate SlPAPs and SlPTs in root and shoot tissue of SlPAP26b silenced lines, under LP condition and in the shoot under HP condition.* ***(C-D)*** *Western blot analysis of SlSPX2 using non-native PAGE in silenced and unsilenced plants under HP and LP conditions.*

2.8.6. *SlPAP26b* promoter is not a direct target of SlPHR1/L1

To check whether the SPX-PHR module transcriptionally regulates *SlPAP26b*, we transiently silenced *SlSPX1/2* and *SlPHR1/L1* separately. Silencing resulted in a more than 50 % decrease in transcript levels of both SlSPXs and SlPHRs **(Fig. 32A, B)**. Because the promoter of *SlPAP26b* harbored one P1BS element, it is a likely target of the master regulators of PSR, SlPHR1/L1 transcription factors. At first, we checked the expression of *SlPAP26b* in *SlSPX1/2* and *SlPHR1/L1* double silenced seedlings. We did not find any change in the mRNA abundance of *SlPAP26b* in these seedlings compared to their unsilenced EV control **(Fig. 32D, E)**. Next, we studied the transcriptional activation of the *SlPAP26b* promoter by SlPHR1/L1 in *N. benthamiana*. GUS assay was performed for the *SlPAP26b* promoter activity with and without SlPHR1/L1 in the co-infiltrated leaves. The negligible GUS activity of the SlPAP26b promoter in the presence of SlPHRL1/L2 suggested no binding of these TFs to the P1BS element of the SlPAP26b promoter, hence no transactivation of *GUS* gene **(Fig. 32C)**. We further quantified the GUS by MUG assay, where no fluorescence was noticed in the leaves co-infiltrated with constructs, pCAMBIA1302-SlPHR1/L1+ p19 and pCAMBIA1391z-SlPAP26b + p19. At the same time, in the positive control, SlPAP15, resulted in higher fluorescence **(Fig. 32F)**. Non-activation of *SlPAP26b* promoter activity by SlPHR1/L1 in these experiments and unchanged transcript levels in *SlSPX1/2* and *SlPHR1/L1* silenced plants indicate transcription regulation of *SlPAP26b* independent of these genes.

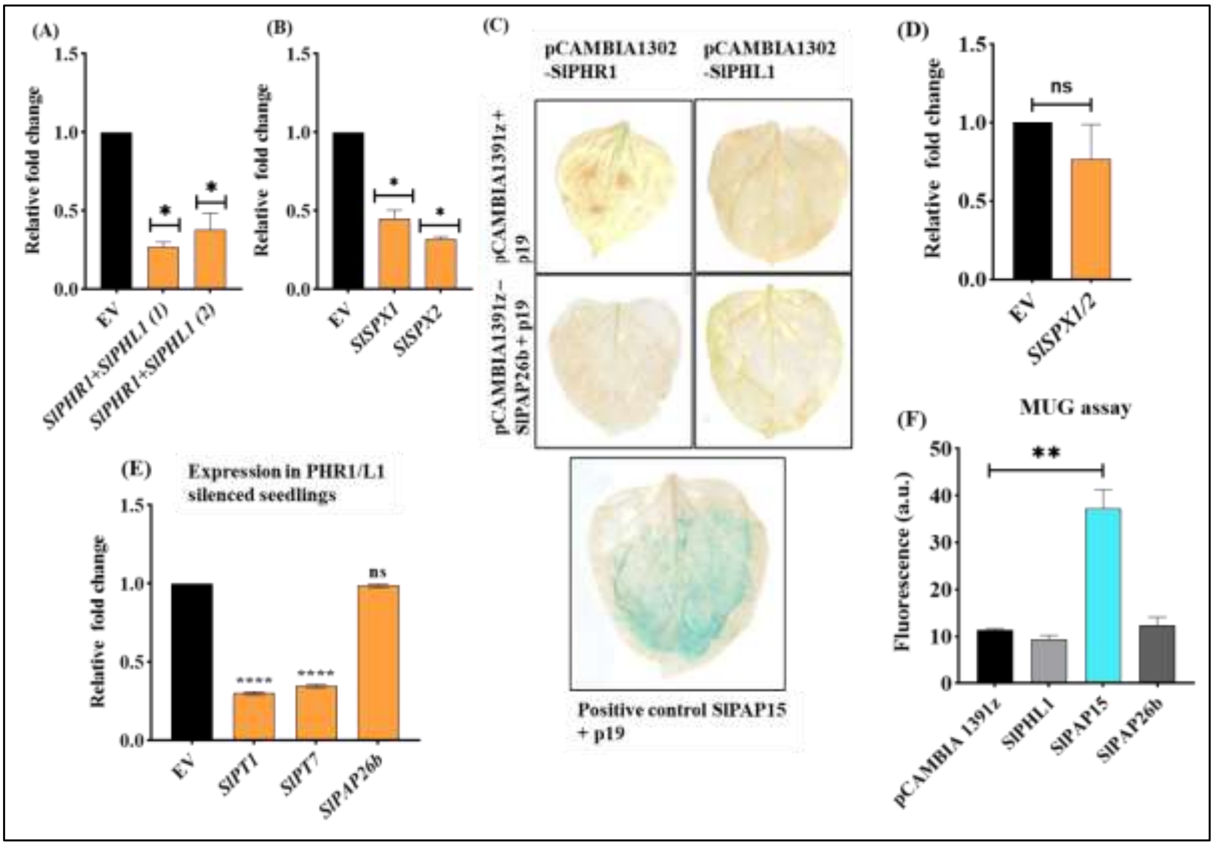


Fig 32: Transient transcriptional activation of pSlPAP26b:GUS promoter via binding of SlPHR1/L1 ***(A)*** *Confirmation of SlPHR1 and SlPHL1 silencing in SlPHR1 + SlPHL1 double silenced seedlings.* ***(B)*** *Confirmation of SlSPX1 and SlSPX2 silencing in SlSPX1 + SlSPX2 double silenced seedlings.* ***(C)*** *GUS assay for SlPAP26b promoter activity with SlPHR1 and SlPHL1 homologs.* ***(D)*** *Transcripts level of SlPAP26b in SlSPX1 + SlSPX2 double silenced seedlings.* ***(E)*** *Transcripts level of SlPAP26b in SlPHR1 + SlPHL1 double silenced seedlings. SlPT1 and SlPT7 acted as controls* ***(F)*** *Quantification of GUS using MUG assay. Error bars indicate standard deviation.*

2.9. Functional characterization of *SlPAP15*, a root-preferential PSI gene

RNA seq data showed *SlPAP15* expression to be more limited to root and to some extent in the flowering stage. RT-qPCR further confirmed its higher mRNA abundance in the root and floral tissues **(Fig. 33A, B)**. Like *SlPAP17b* and *SlPAP26b, SlPAP15* is rapidly induced upon Pi starvation and exogenous sucrose supply under LP conditions. During the recovery experiment, ~ 95 % of the transcripts recovered within 15 min of Pi resupply **(Fig. 33C-E)**.

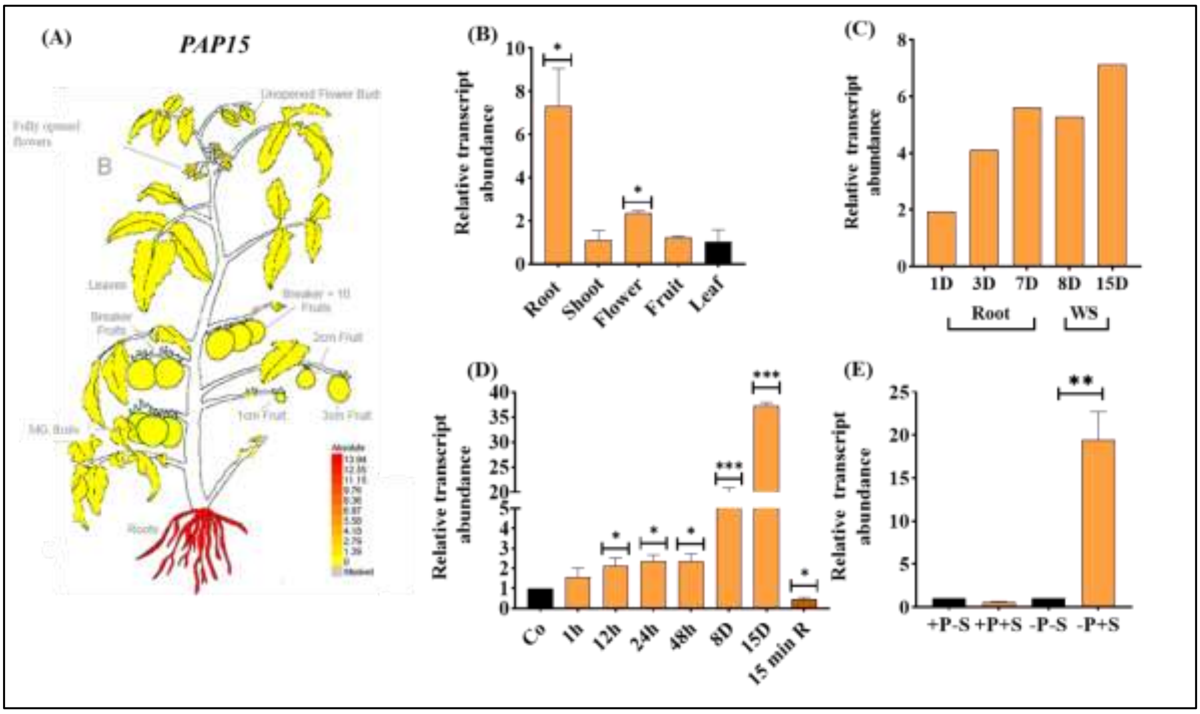


Fig 33: Expression analysis of SlPAP15 (A-B) *Expression analysis of SlPAP15 gene at different development stages using Multi-Plant eFP Browser 2.0 – BAR tool and RT-qPCR* ***(C)*** *Expression of SlPAP15 in different tissues and at different time points in RNA-sequencing data.* ***(D)*** *Time-course kinetics and recovery of SlPAP15 upon Pi starvation and Pi resupply.* ***(E)*** *The expression level of SlPAP15 upon sucrose treatment in both HP and LP conditions.*

2.9.1. *SlPAP15* has close sequence similarity at the protein level in Arabidopsis and rice

The multiple sequence alignments of *SlPAP15* and its close Arabidopsis and rice homologs showed good sequence similarity with rice (79.89 %) and Arabidosis (83.78 %) members suggesting conserved functions of three proteins **(Fig. 34A, B)**. The presence of REKA motifs indicates that the gene has phytase activity.

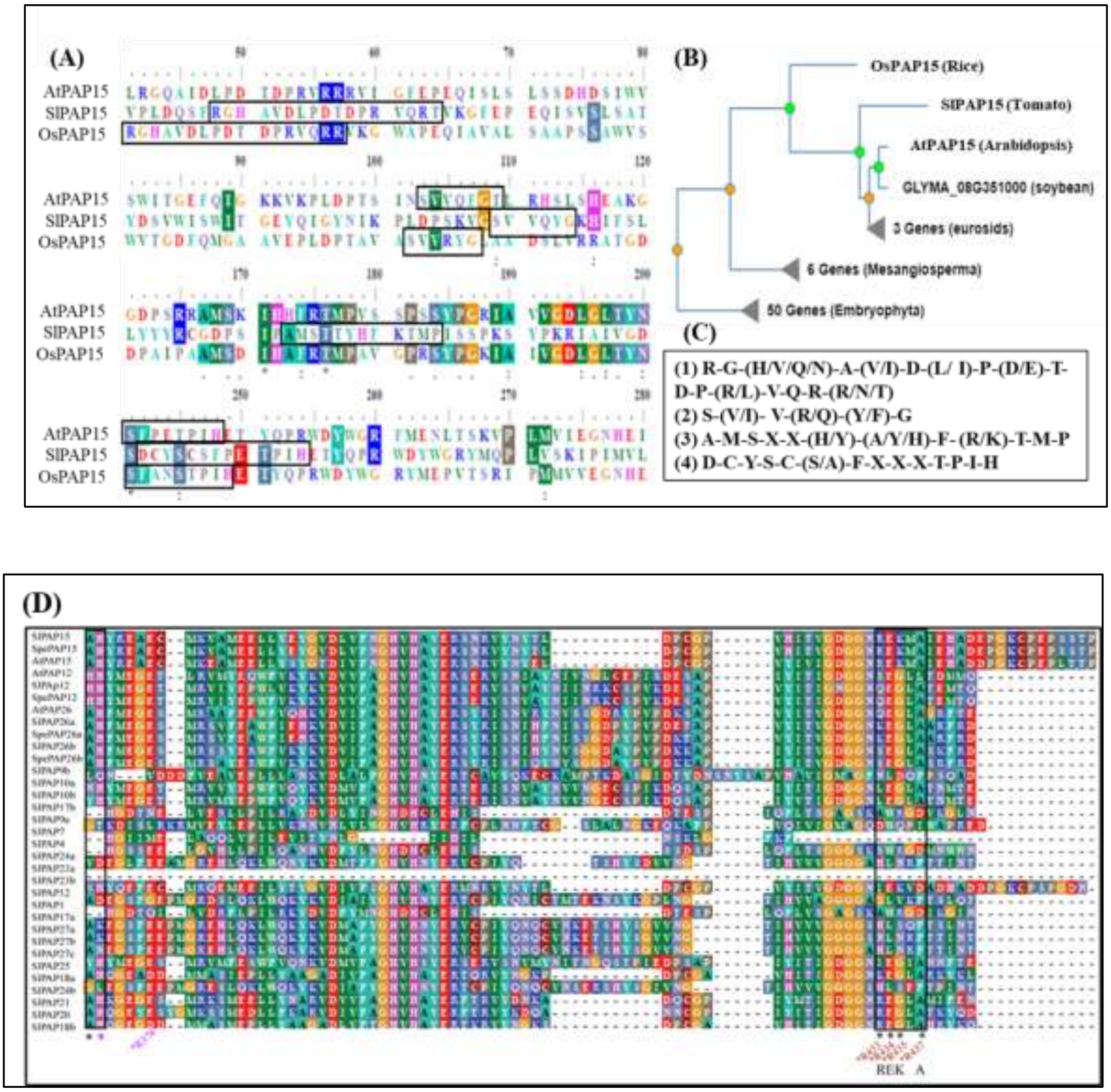


Fig 34: Insilico study of SlPAP15 in its close homolog in Rice and Arabidopsis (A) *Multiple sequence alignment of SlPAP15, AtPAP15 and OsPAP15 protein sequences* ***(B)*** *Phylogenetic analysis of SlPAP15 using PhyloGenes tool from SGN* ***(C)*** *SlPAP15 contains five conserved motifs.* ***(D)*** *Multiple sequence alignment resulted in the presence of REKA motif in SlPAPs and in their close orthologue. The lysine residue at R558 and a conserved motif "("REKA"") in the C-terminal region, positioned between R1064-R1068, have been found necessary for phytate binding to the plant SlPAP. Selected amino acid residues known to influence the substrate preference, especially phytate, of plant PAPs, according to Feder et al. (2020).*

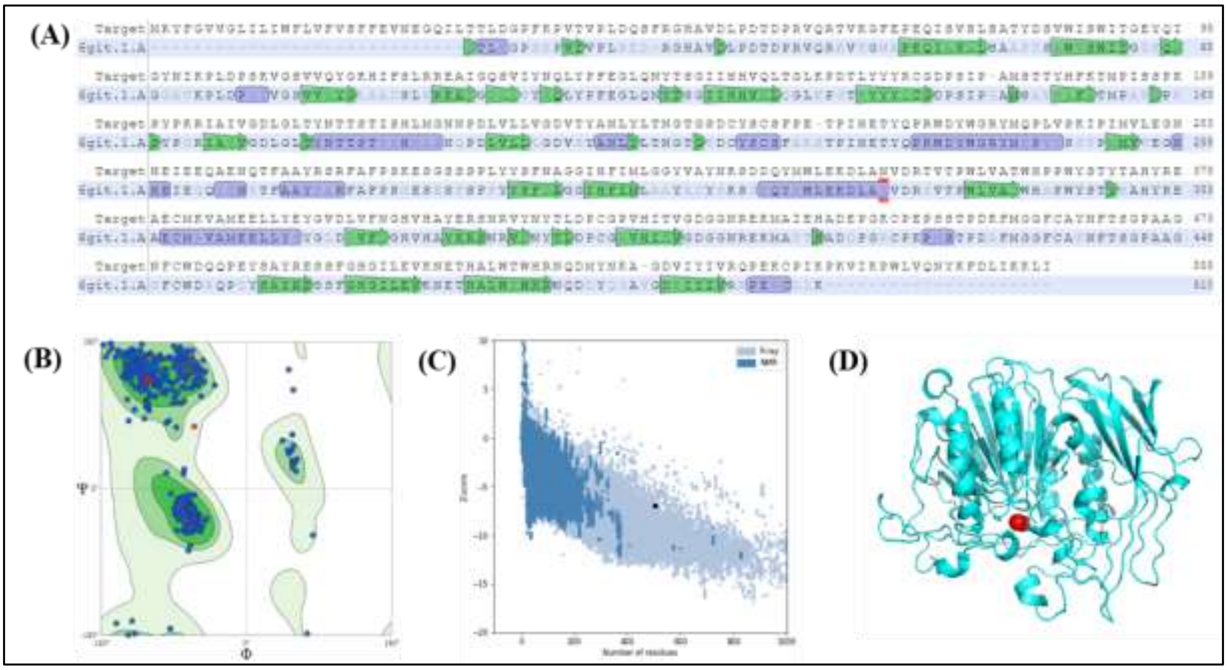


Fig 35: Homology modeling of SlPAP15 protein. (A) SlPAP15 *protein sequence alignment to Triticum aestivum purple acid phosphatase (PDB:6GIT) template has 74.8 % identity and 91 % query coverage, leaving only N-terminal and C-terminal overhang regions.* ***(B)*** *phi-psi torsional constraints were validated using the Ramachandran plot.* ***(C)*** *Overall modeled structure quality was assessed using PROSA server.* ***(D)*** *Modeled 3-D structure of* SlPAP15 *protein based on 6GIT template using Modeller v9.20. Functionally important, two iron atoms (red color) are also incorporated into the model. These three validation methods confirmed the high quality of the modeled* SlPAP15 *structure.*

Due to its root-preferential transcripts accumulation, predicted secretory and PSI nature, and presence of the characteristic REKA motif (R433-R437) in the complete sequence, SlPAP15 was selected for further examination**.** Before homology modeling, 27 amino acid truncation at N-terminal was made at R406-R410 in the modeled protein to study its structure **(Fig. 35A-D)**. Its preferential substrate was identified by performing docking studies with different substrates **(Fig. 36)**. Of the four substrates used in this analysis, only ATP and ADP were successfully docked with SlPAP15. Surprisingly, neither phytate (IHP) nor phosphoenolpyruvate (PEP) was found to interact with any of the Fe

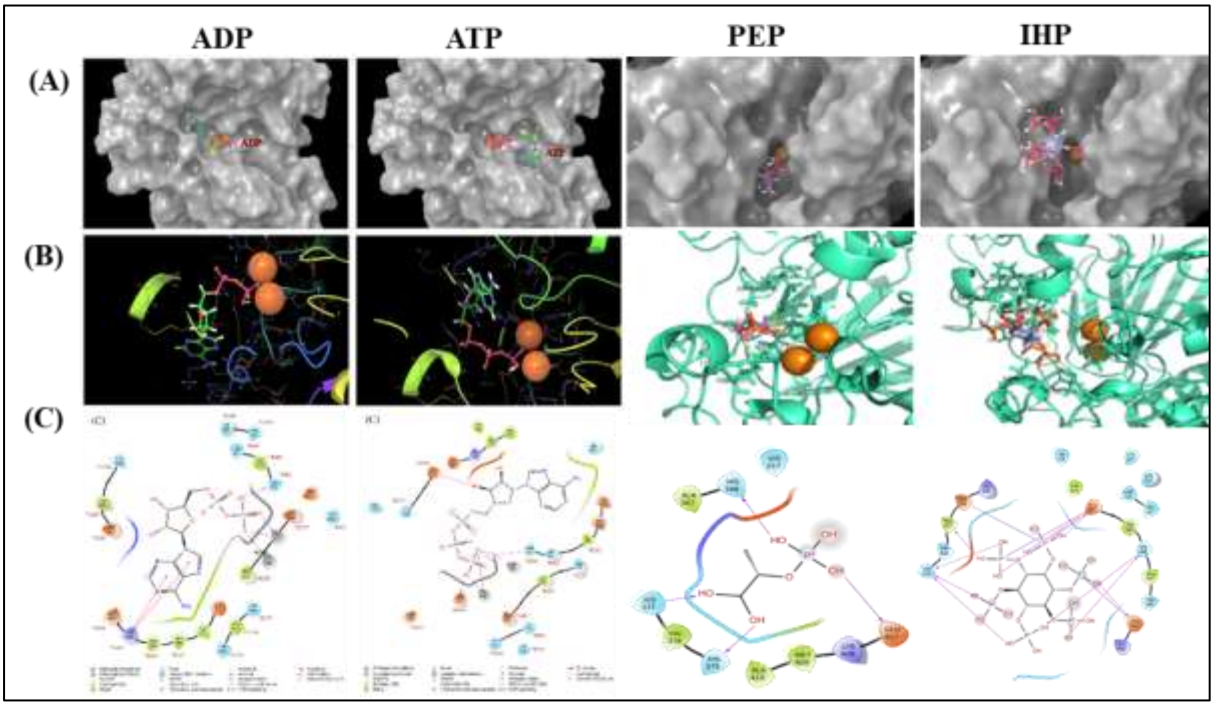


Fig 36: Docking of ATP, ADP, PEP and IHP ligand in the active site of SlPAP15 protein. ***(A)*** *Surface and stick representation SlPAP15-ATP complex. Two Fe(III) atoms are shown as orange spheres.* ***(B)*** *Interaction of ADP, ATP, PEP and IHP (stick representation) at the active site of SlPAP15 (cartoon representation). Two functionally important Fe(III) are shown as orange spheres.* ***(C)*** *A 2-D ligand interaction map of the ADP/ATP/PEP/IHP-SlPAP15 complex.*

metal ions of SlPAP15 and, hence, could not be docked. Although, we noticed that these two substrates were also reaching the active site when allowed to dock, even without a proper interaction between the substrates and the metallic dinuclear iron center **(Fig. 36A, B)**. The active sites in the case of ADP and ATP interaction were found to be enriched by basic amino acid residues. At least one residue of the REKA domain was found to interact with ADP and ATP **(Fig. 36C)**. While lysine residue of the REKA domain (R408) facilitated the Pi-cation interaction with ADP, glutamate (R407) facilitated such interaction with ATP by forming H-bond **(Fig. 36C)**. Asparagine (R283) and histidine (R284) form a conserved NH domain and are present in the active site in both ATP and ADP **(Fig. 36C)**. Other residues, such as glutamate (R378), asparagine (R283) with ATP and histidine (R402) with ADP, also helped their interaction through H-bond. In both the docked proteins, a Tyrosine 203 (R230) residue was found close to the dinuclear iron center. Three more histidine residues, positioned at R365, R402, and R404, were also found to be present in both docked proteins **(Fig. 36C)**.

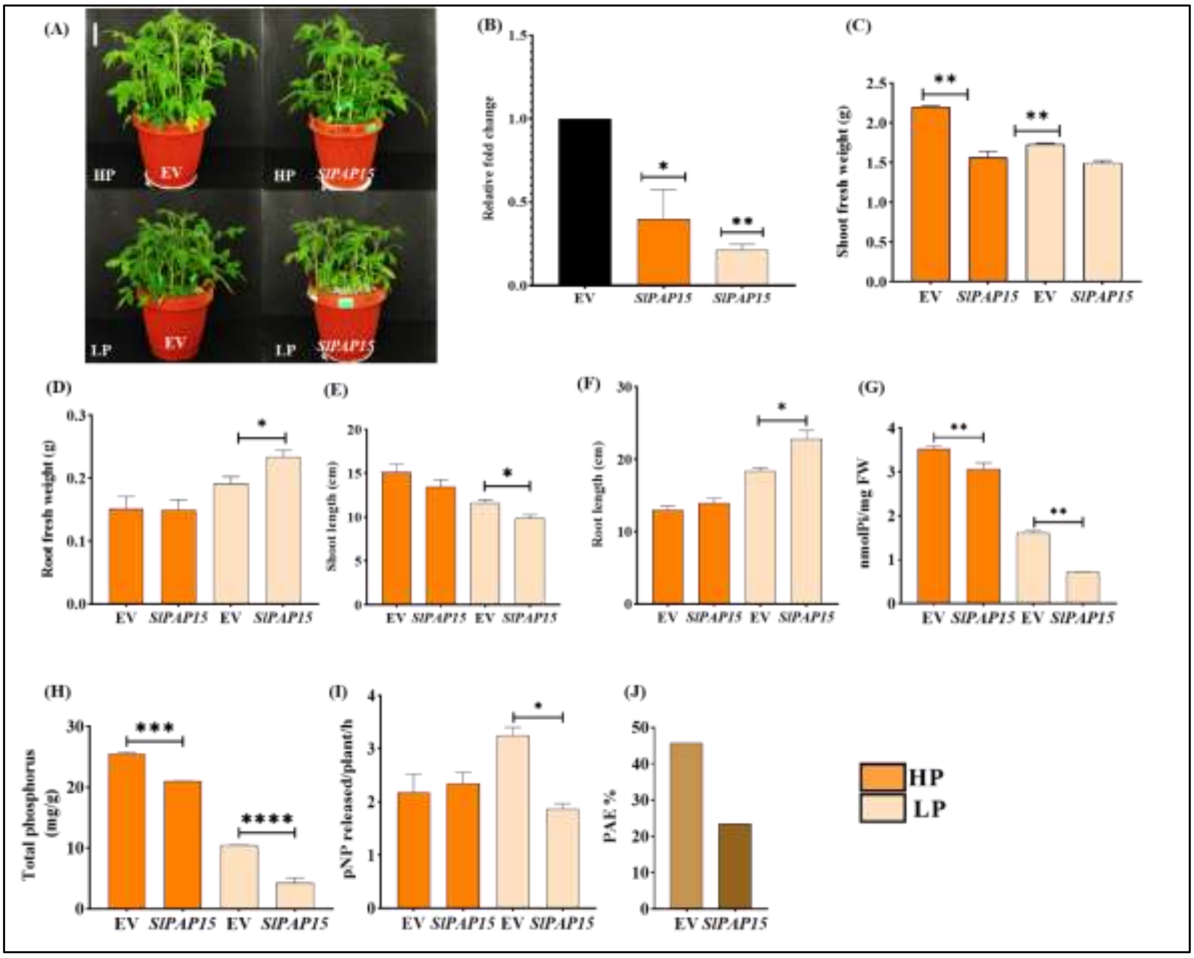


***Fig 37: Silencing of SlPAP15 using VIGS (A)** Growth phenotype of SlPAP15 silenced plants and their EV-controls **(B)** Confirmation of SlPAP15 silencing at the transcript level **(C-D)** Shoot and root fresh weight **(E-F)** Shoot and root length **(G-H)** Total soluble Pi content and total phosphorus in SlPAP15 silenced tomato seedlings under HP and LP conditions, respectively. **(I-J)** SAP and PAE (phosphorous acquisition efficiency) in SlPAP15 silenced seedlings and its EV-control under HP and LP conditions.*

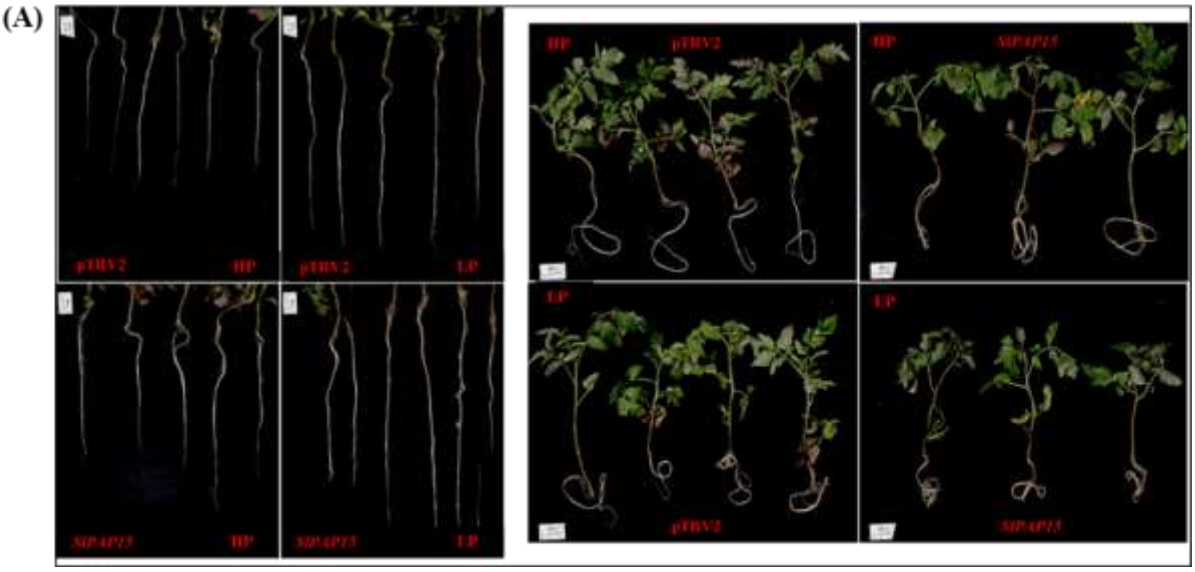


Fig 38: Root and shoot phenotype of SlPAP15 silenced and EV seedlings.

2.9.2. VIGS-based characterization of *SlPAP15*

As described earlier, we observed good efficiency of around 80 % in our positive control *SlPDS*-silenced plants. While silencing of this gene resulted in ~65 % lesser *SlPAP15* transcripts in the HP condition, a more robust silencing with ~ 85 % lesser transcripts was found in the LP condition than in their respective control EV plants **(Fig. 37B)**. At the phenotypic level, although we did not see much change in the silenced and EV seedlings in HP condition, a more severe phenotype in *SlPAP15* silenced seedlings was observed in LP conditions **(Fig. 38)**. Shoot fresh weight significantly decreased in the silenced seedlings under HP and LP conditions. In contrast, we noticed increased root fresh weight in the silenced seedlings under LP conditions **(Fig. 37C, D)**. As evident from shoot length, the change in biomass can be due to the stunted plant growth observed in silenced seedlings under LP condition, but no such change was observed in HP condition **(Fig. 37E)**. The primary root length increased in silenced seedlings than EV seedlings under LP conditions **(Fig. 37F)**. The soluble Pi content and total phosphorus also decreased in *SlPAP15* silenced seedlings than EV controls under HP and LP conditions **(Fig. 37G, H)**. The silenced plants also exhibited reduced secretory APase activity and PAE, which were reduced by ~50 %, than their control counterparts under the LP condition **(Fig. 37I, J)**.

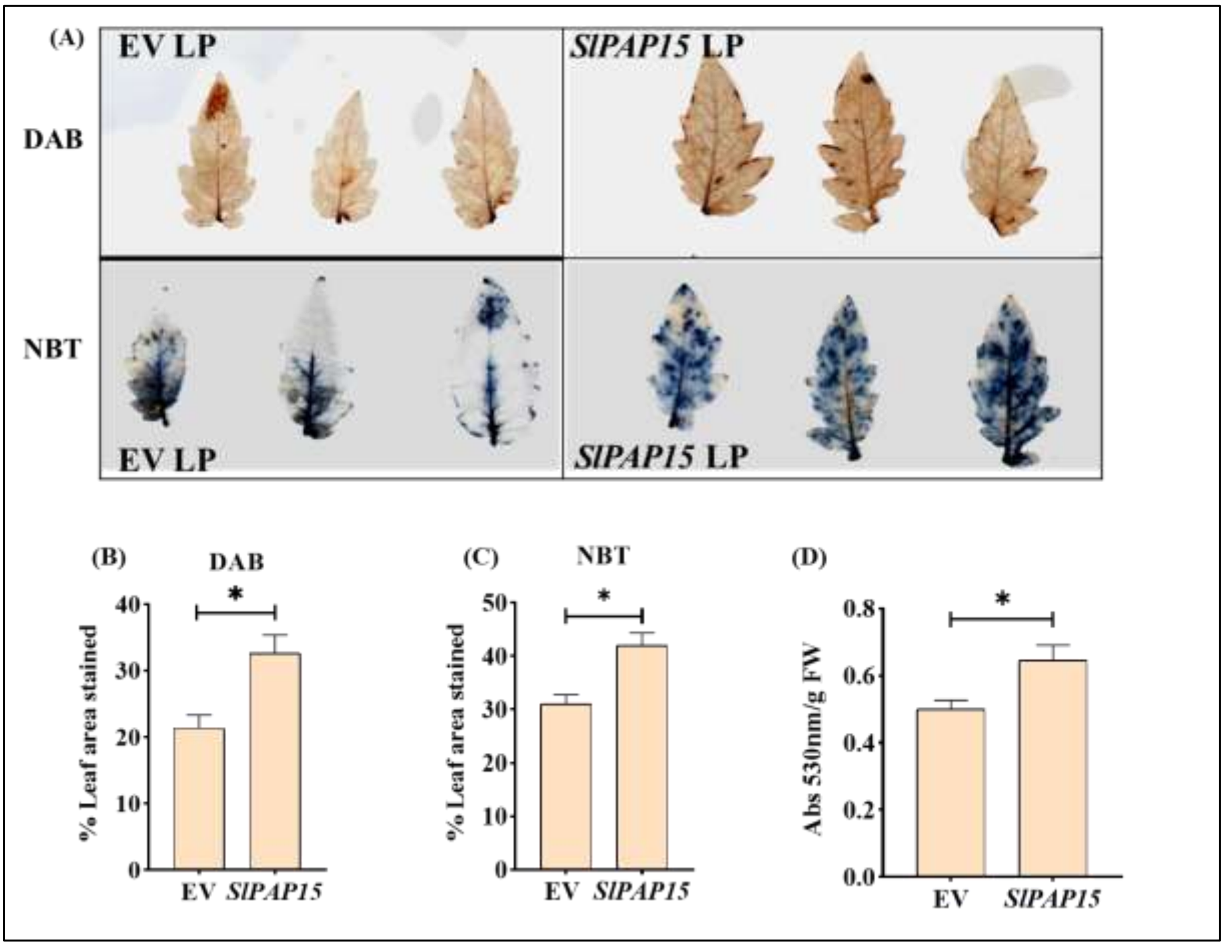
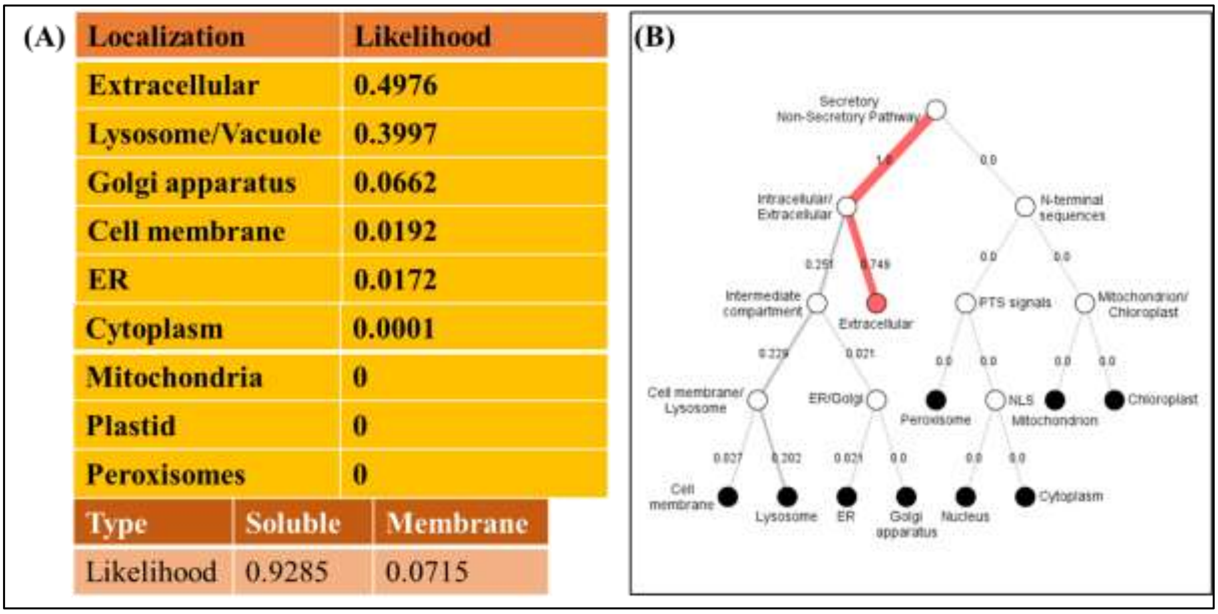


***Fig 39: ROS accumulation was affected in silenced seedlings under LP condition (A)** Histochemical staining of ROS using NBT/DAB staining in the leaves of SlPAP15 silenced and EV seedlings **(B-C)** Quantification of ROS using ImageJ software **(D)** Total anthocyanins content in SlPAP15 silenced seedlings under LP condition.*

2.9.3. Silencing of *SlPAP15* affects ROS accumulation and total anthocyanin content

ROS levels change in seedlings upon phosphate starvation. Many enzymes are responsible for either scavenging the generated ROS or reducing the production of ROS. NADPH oxidase is a class of enzymes that helps generate ROS, while reports have suggested the antioxidant role of purple acid phosphatase. Hence, we were interested in checking whether *SlPAP15* silenced seedlings had altered ROS levels. NBT/DAB staining in the silenced seedlings resulted in higher ROS accumulation under LP conditions **(Fig. 39A-C),** indicating the severity of Pi starvation stress in these plants. We also noticed higher anthocyanins content in the silenced seedlings than in EV controls under LP conditions **(Fig. 39D)**.


(A)

Localization	Likelihood
Extracellular	0.4976
Lysosome/Vacuole	0.3997
Golgi apparatus	0.0662
Cell membrane	0.0192
ER	0.0172
Cytoplasm	0.0001
Mitochondria	0
Plastid	0
Peroxisomes	0

Type	Soluble	Membrane
Likelihood	0.9285	0.0715

Fig 40: (A-B) SignalP version 5.0 was used to determine the localization and nature of the protein sequence.

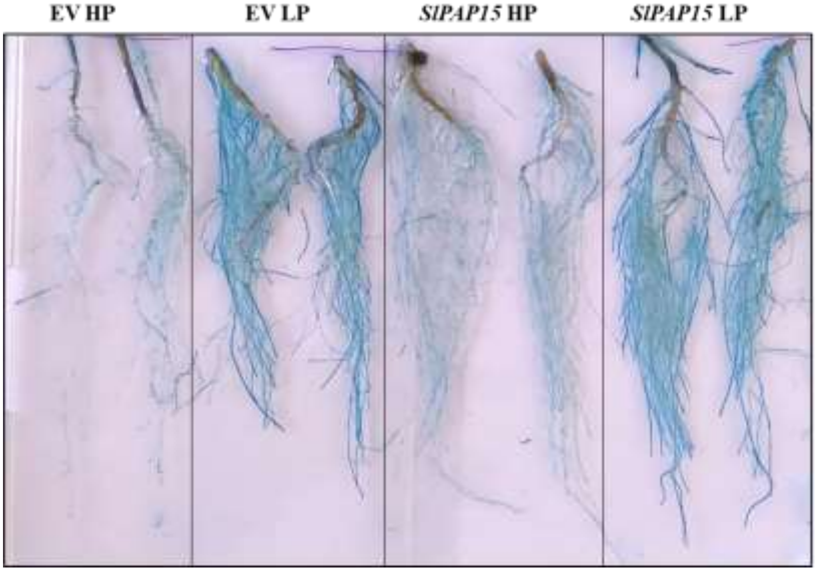


Fig 41: BCIP staining for root surface associated APase activity.

2.9.4. Confirmation of *SlPAP15* secretory nature using BCIP

I*n silico* analysis by signal prediction 5.0 predicted this gene to be extracellular/secretory/soluble in nature **(Fig. 40A, B)**. The lack of an RGD binding motif in its protein sequence also confirms this gene does not adhere to the membrane. We also did histochemical staining to determine any change in root surface-associated APase activity in the silenced and EV control roots. We found no visible difference in *SlPAP15* silenced lines compared to that of unsilenced EV-control roots in HP and LP conditions **(Fig. 41).**

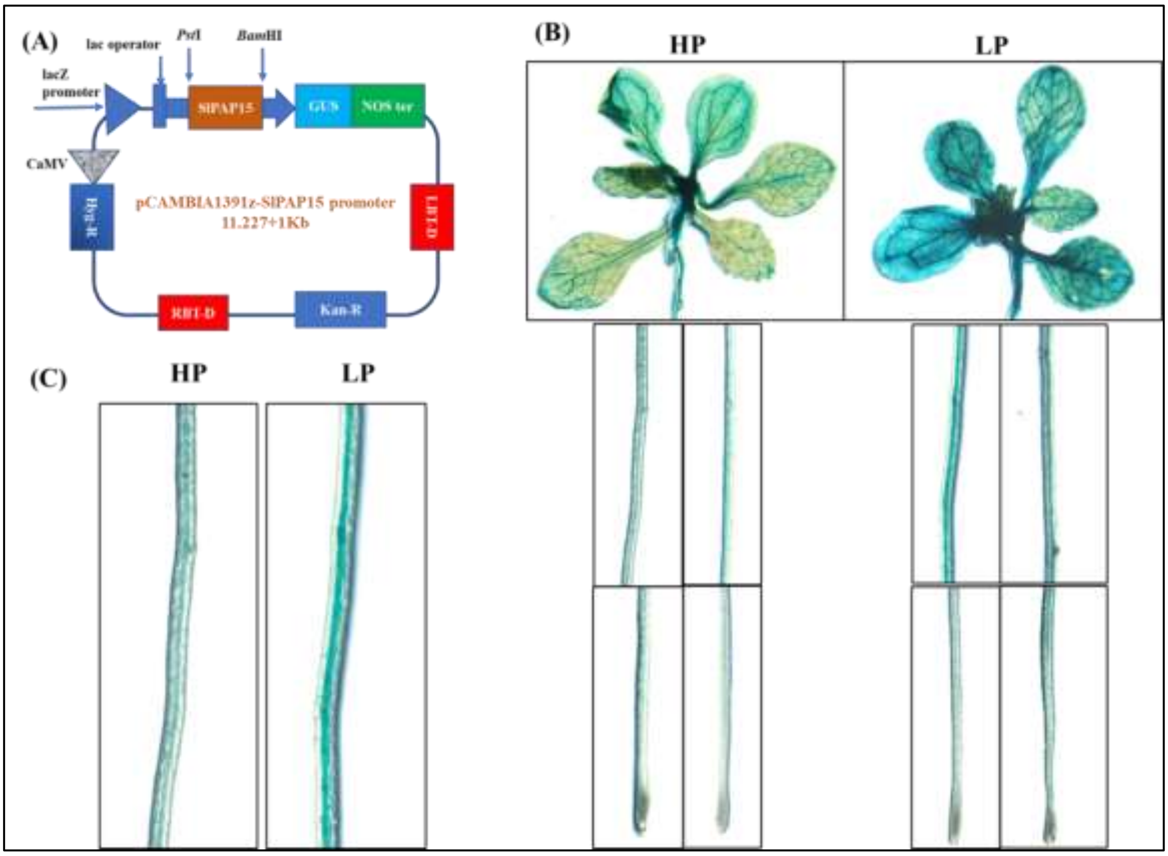


Fig 42: SlPAP15 promoter characterization in stable Arabidopsis transgenic lines ***(A)*** *Cloning of SlPAP15 promoter in pCAMBIA1391Z:GUS binary vector* ***(A)*** *Representation of 1-kb promoter region of SlPAP15 in pCAMBIA1391z:SlPAP15-GUS seedlings****. (B)*** *Aerial part* ***(C)*** *Roots. SlPAP15 promoter activity was checked in 15-D-old Arabidopsis seedlings of transcriptional fusion transgenic line on HP/LP ½ MS solid media.*

2.9.5. Characterization of *pSlPAP15:GUS* in stable Arabidopsis lines

Due to the PSI nature of *SlPAP15* and the presence of P1BS elements, we cloned 1-kb promoter region of *SlPAP15* harboring two P1BS elements in pCAMBIA1391z vector **(Fig. 42A)**. Stable transcriptional reporter seven Arabidopsis lines in Col-0 background were generated. Promoter activity was analyzed using GUS histochemical staining, which showed higher activity of this promoter in both roots and leaves under the LP condition compared to the HP condition. A more intense GUS staining was noticed in the roots and leaves of these lines under LP conditions, supporting its PSI nature **(Fig. 42B, C)**. Interestingly, *SlPAP15* promoter showed substantial activity even under HP conditions, indicating its important role in Pi homeostasis even under Pi-sufficient conditions.

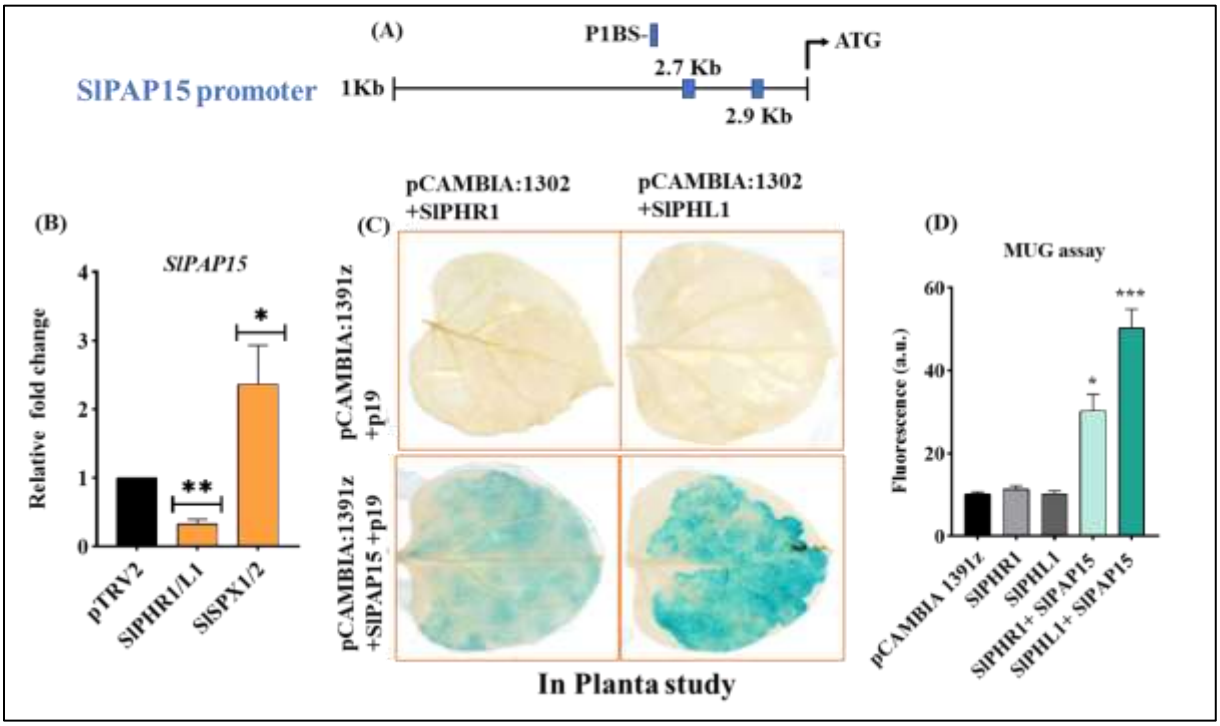


Fig 43: In-plants transient transcriptional activation of pSlPAP15: GUS promoter via binding of SlPHR1/L1 (A) *Two P1BS (GNATATNC) elements are present in 1-kb upstream sequence of SlPAP15* ***(B)*** *Transcript accumulation of SlPAP15 in SlPHR1 + SlPHL1 and SlSPX1 + SlSPX2 silenced seedlings* ***(C)*** *GUS assay for* SlPAP15 *promoter activity was performed separately with SlPHR1 and SlPHL1 homologs.* ***(D)*** *Quantification of GUS using MUG assay. Error bars indicate standard deviation.*

2.9.6. SlPAP15 promoter is a direct target of SlPHR1/L1

The presence of two P1BS elements in the 1-Kb upstream promoter region of *SlPAP15* indicates it to be a possible direct target of SlPHR1/L1 **(Fig. 43A)**. To confirm this, we first checked the mRNA levels of *SlPAP15* in *SlSPX1*/2 and *SlPHR1*/L1 double-silenced seedlings **(Fig. 43B)**. Down-regulation of *SlPAP15* in SlPHR1/L1 double silenced seedlings and its upregulation in the *SlSPX1*/2 silenced seedlings supported our notion that this gene is under the direct regulation of SPX-PHR regulatory module **(Fig. 43B)**. To reconfirm the above results, we checked the transcriptional activation of *SlPAP15* promoter by SlPHR1/L1 in *N. benthamiana* **(Fig. 43C)**. GUS assay was performed for the *SlPAP15* promoter activity with and without SlPHRL1/L2 TFs. The enhanced GUS activity of the *SlPAP15* promoter in the presence of SlPHRL1/L2 suggested the activation of this promoter by both TFs **(Fig. 43D)**. More robust activation of *SlPAP15* promoter activity was observed with SlPHL1 compared to PHR1, indicating a more prominent role of this TF in activating the *SlPAP15* gene in Pi-deficient seedlings.

2.9.7. Generation and functional characterization of *Slpap15*^{CR} KO lines under Pi deficiency

We next generated CRISPR/Cas9-based gene-edited knockout (KO) stable mutant tomato lines of the *SlPAP15* gene. For this, we first cloned the guide in pFASTRK vector, followed by sequence confirmation via sanger sequencing **(Fig. 44A-D)**. We generated six stable knockout lines, including *Slpap15*^{CR1}, *Slpap15*^{CR2}, *Slpap15*^{CR3}, *Slpap15*^{CR4,} *Slpap15*^{CR5,} and *Slpap15*^{CR6}. Two lines *Slpap15*^{CR1}, *Slpap15*^{CR2} were confirmed by Sanger sequencing. Both KO lines showed the insertion of 1-base pairs, which disrupted the conserved motif Pur_ac_phosph_N domain in this protein. The loss of the conserved domain suggested a severely altered function of the mutated protein in the KO background **(Fig. 46B-D)**. We ruled out any off-target editing by conforming the editing in the closest predicted sequence of gRNA via Sanger sequencing.

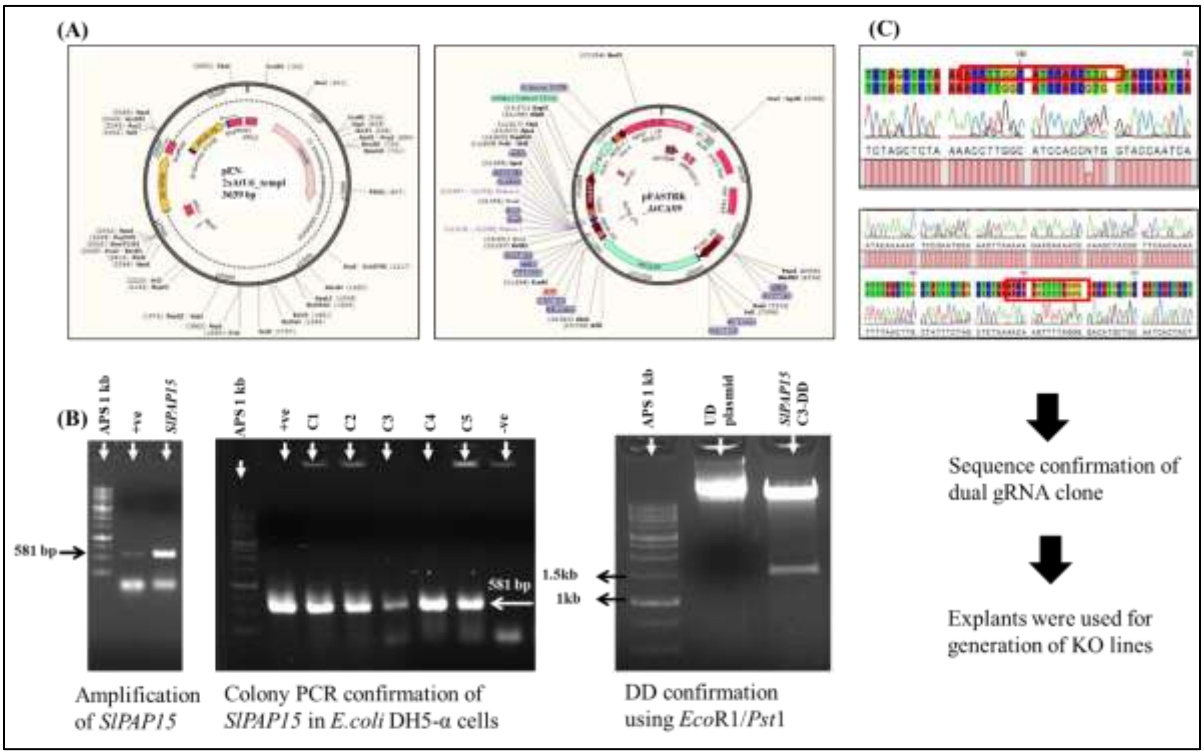


Fig 44: Construction of CRISPR pFAST-RK- SlPAP15 expression vector (A) *Vectors (pEN-2xU6 and pFASTRK) used for CRISPR cloning of SlPAP15 using dual guide RNA approach.* ***(B)*** *Amplification, colony PCR confirmation, and double digestion of final confirmed clone using EcoRI and PstI.* ***(C)*** *Sequence confirmation using Sanger sequencing.*

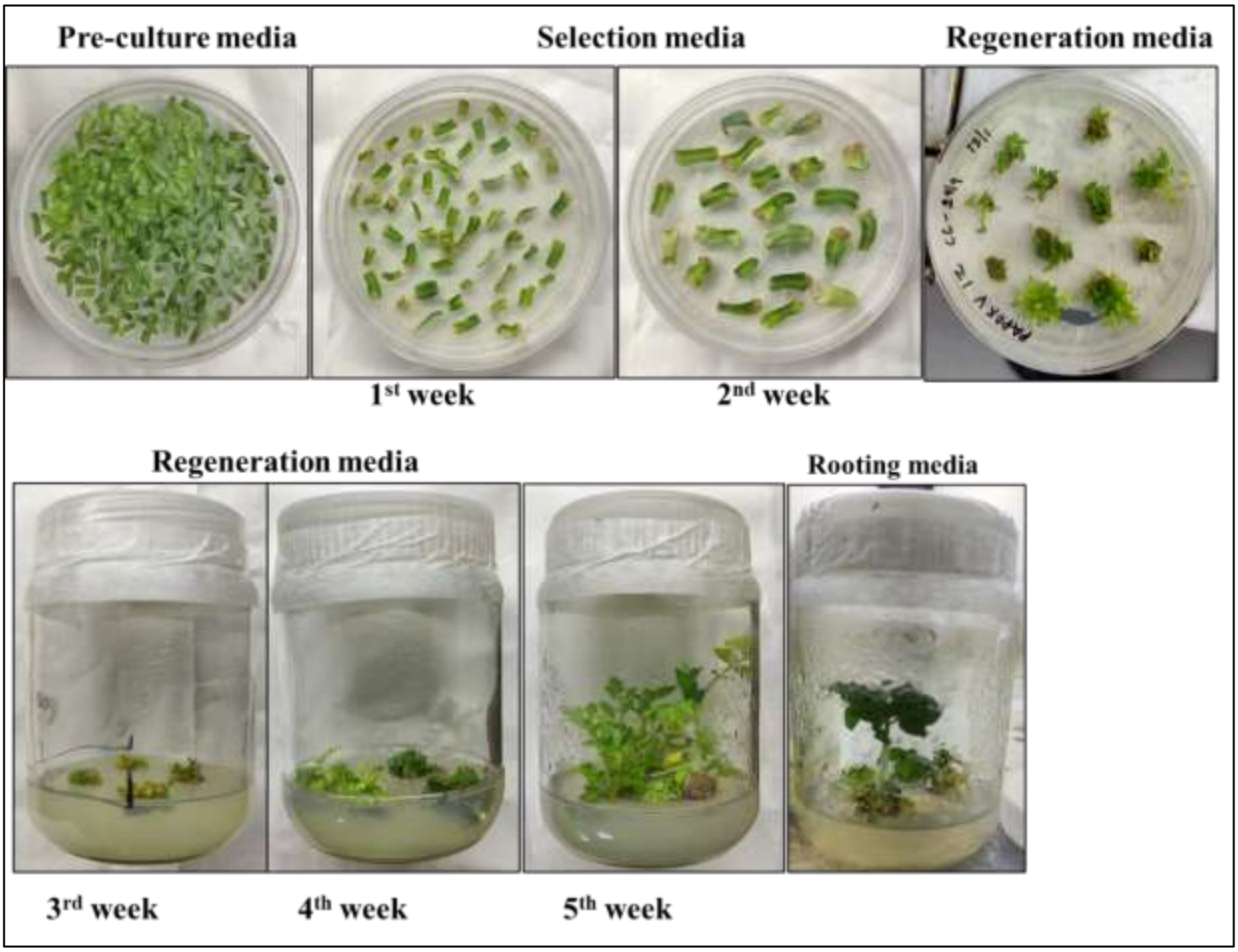


Fig 45: Different stages of plant tissue culture to generate SlPAP15- edited and OX lines in tomato.

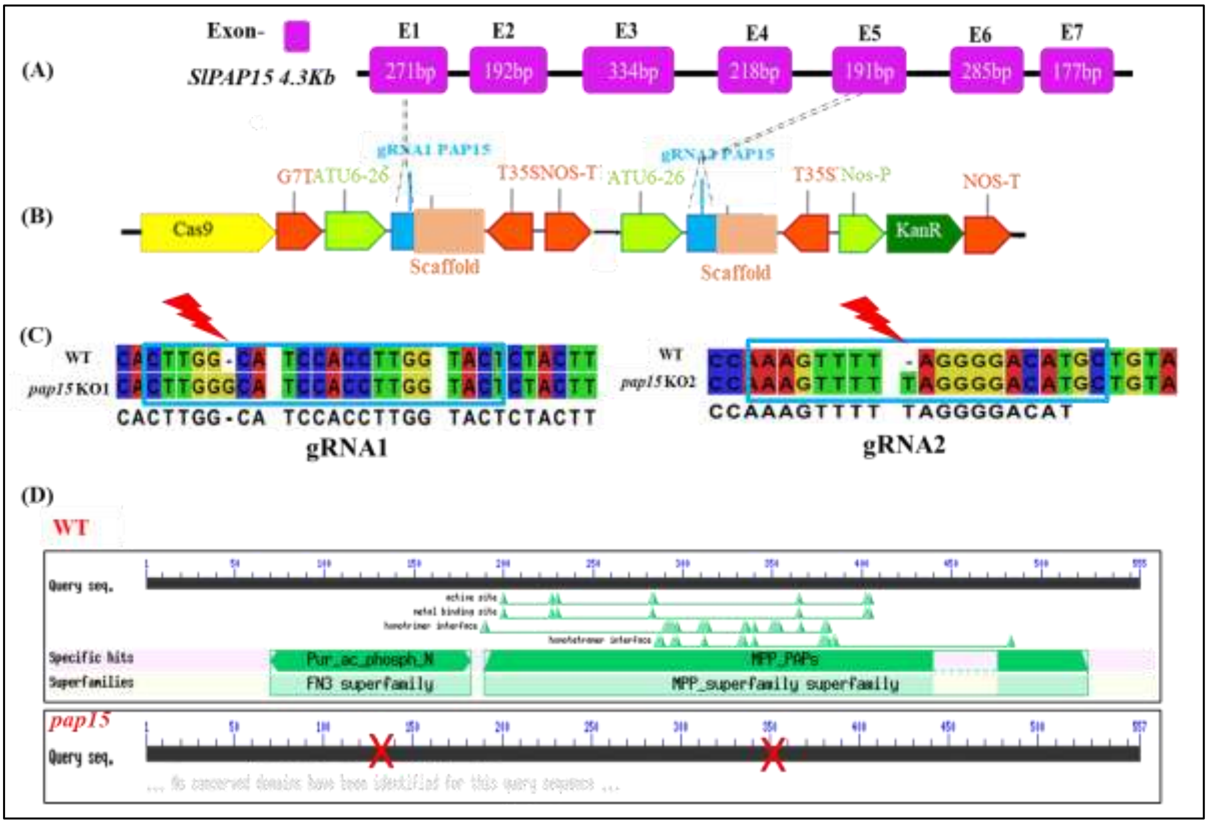


Fig 46: Generation of Slpap^{CR} knockout tomato lines. ***(A)*** *Exon-1 and Exon-5 were targeted for gRNA designing and editing* ***(B)*** *Vector map of pFASTRK-AtCas9-AtU6.* ***(C)*** *One base pairs insertion was observed in the CRISPR-edited KO lines* ***(D)*** *Domain analysis of SlPAP15 protein sequences in WT and the edited KO lines. One base pairs insertion in the SlPAP15 coding sequence disturbed the conserved domains in this protein.*

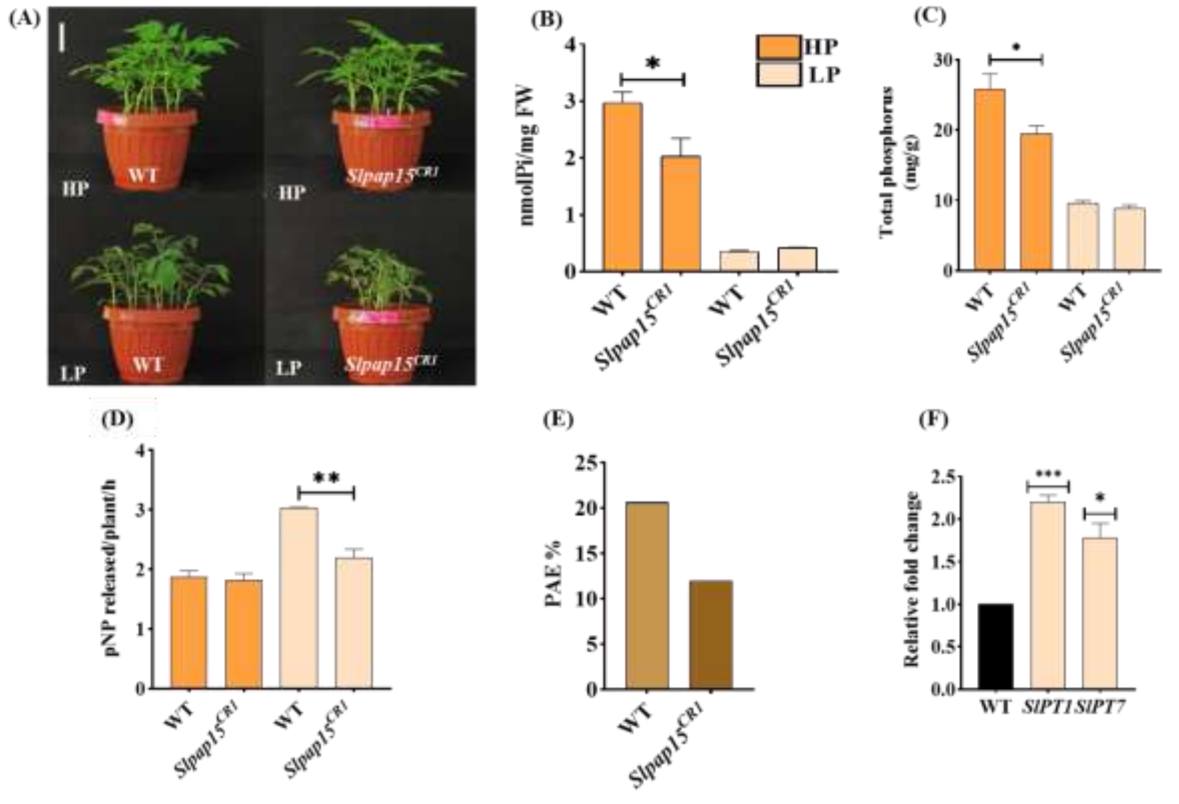


Fig 47: Characterization of Slpap15^{CR1} KO plants under HP and LP conditions (A) *Seedlings phenotype of wild type (WT) and Slpap15^{CR1} KO lines under HP and LP condition* ***(B)*** *Total soluble Pi content* ***(C)*** *Total phosphorous content* ***(D)*** *Secretory acid phosphatase activity* ***(E)*** *PAE (Phosphorus acquisition efficiency) under LP condition.* ***(F)*** *Expression of SlPTs in the root of Slpap15^{CR1} KO lines under LP conditions.*

Similar to the VIGS results, stunted plant growth was observed in *Slpap15^{CR1}* KO line, mainly under LP conditions **(Fig. 47A)**. The KO line plants also showed decreased soluble Pi and total phosphorus content under HP and LP conditions **(Fig. 47B, C)**. SAP & PAE was found to be reduced by ~ 33 % and ~ 50 % respectively, in the case of KO lines under LP condition **(Fig. 47E)**. Interestingly, higher transcripts level of *SlPT1* and *SlPT7* were observed in KO lines than WT seedlings under LP condition **(Fig. 47F)**. Because *in-silico* analysis has predicted phytase activity for *SlPAP15*, we also checked this activity in the KO seedlings **(Fig. 48A)**. Phytase activity was checked in 5-day-old seedlings grown in the presence of organic-P i.e., phytate and inorganic-P, KH_2PO_4. We observed lower phytase activity in the KO lines than WT plants **(Fig. 48B)**.

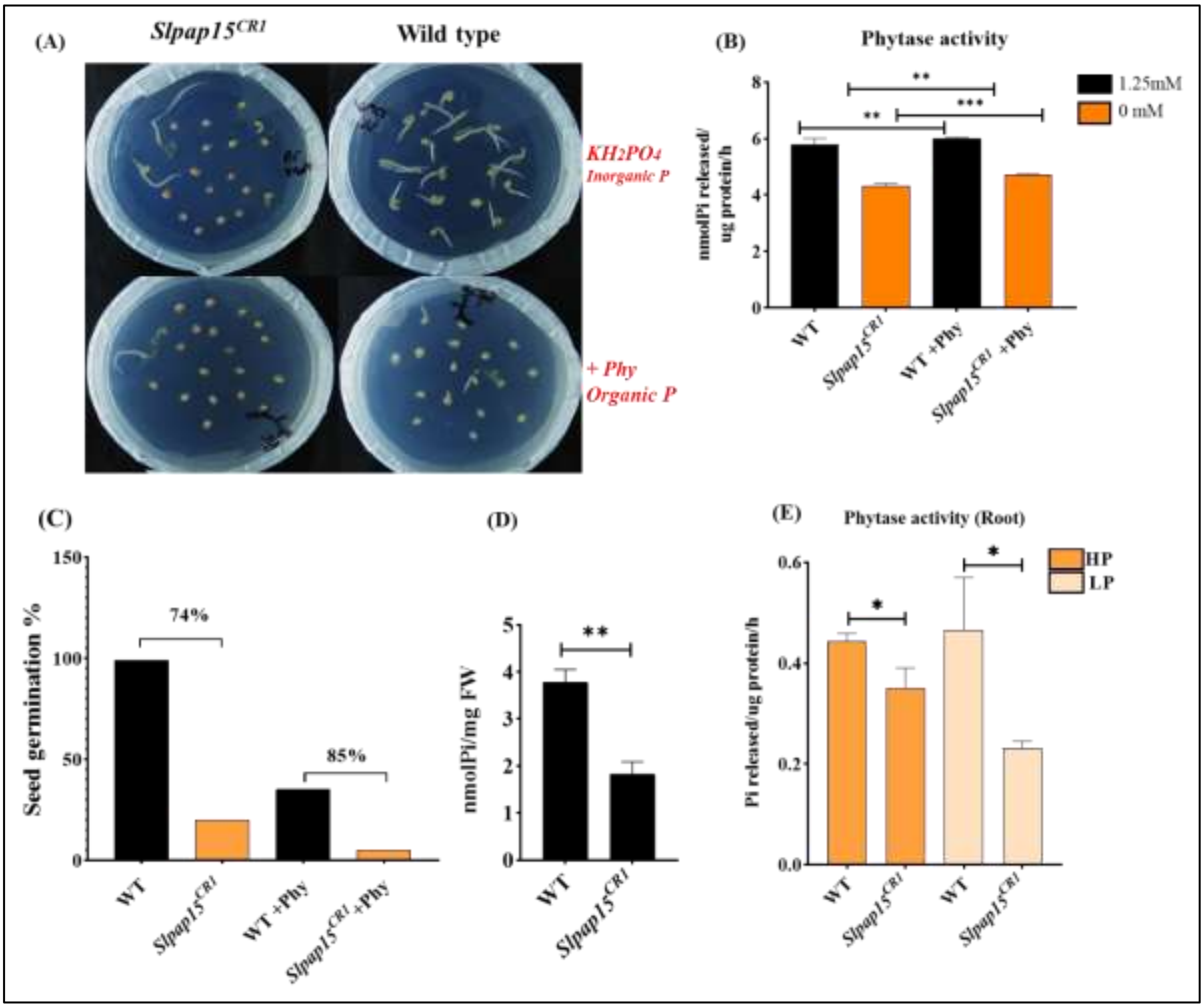


Fig 48: Phytase activity at seedling stage in WT and Slpap15^{CR1} KO seeds. A) *Wild type (WT) and Slpap15^{CR1} KO seeds were grown in the presence of organic (phytate) and inorganic phosphate for 5-D post-transfer in ½X MS media* ***(B)*** *Phytase activity at the seedling stage* ***(C)*** *Seed germination percentage was checked in presence of inorganic (KH_2PO_4) and organic (Phytate) source of Pi.* ***(D)*** *Total soluble Pi content in WT and Slpap15^{CR1} KO lines under Pi-sufficient condition* ***(E)*** *Phytase activity in 30-d-old root under HP and LP conditions. In total 20 seeds from each wild type and KO line was taken for the study.*

Phytase activity remained low even in the root of 30-d-old seedlings under HP and LP conditions **(Fig. 48E)**. Decreased total soluble Pi content in the KO line seedlings indicates seed germination may be affected in the KO lines **(Fig. 48D)**. For this we calculated seed germination percentage, which has reduced to 74 % in the presence of KH_2PO_4 and by 85 % in the presence of phytate **(Fig. 48C)**.

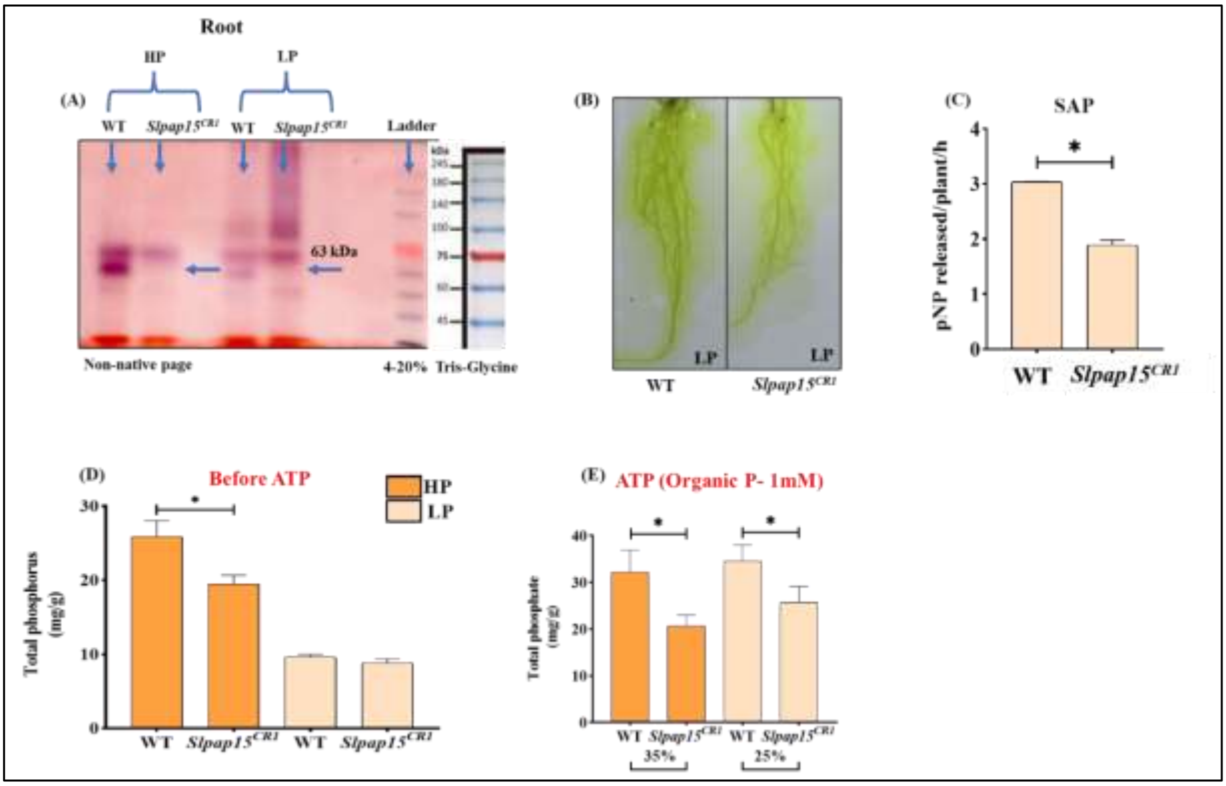


Fig 49: APase activity and total P content in wild type (WT) and Slpap15^{CR1} knockout plants* (A)** *In-gel APase activity in WT and Slpap15^{CR1} knockout line plants grown under HP and LP conditions* ***(B) *Histochemical staining of roots using pNPP as a substrate for plant secretory acid phosphatase activity* ***(C)*** *Quantification of SAP in WT and Slpap15^{CR1} knockout lines* ***(D-E)*** *Total phosphorus content in WT and the KO line seedlings grown for five days after ATP supplementation. The percentage decrease in total P content in seedlings after ATP supplementation under both HP and LP conditions was 35 % and 25 %, respectively.*

Consistent with our VIGS result, the reduced APase activity in *Slpap15^{CR1}* KO seedlings also followed the same trend under LP conditions. This observation was revalidated by in-gel APase assay, where the absence of a band at 63 kDa confirms the diminished activity of SlPAP15 in the KO lines compared to wild type under both HP and LP conditions **(Fig. 49A)**. Histochemical staining using pNPP as a substrate also resulted in subdued activity in the case of KO lines under LP condition, as evident in the quantification results **(Fig. 49B, C)**. Based on the lower P levels in VIGS-silenced and KO lines plants, we anticipated SlPAP15 role in PAE. To substantiate this, we provided external ATP (1mM) and checked the total P content in seedlings of wild type and the KO line. Lower accumulation of total P in the seedlings of the KO line than the wild type in the presence of an exogenously supplied organic-P source affirms its role in P acquisition **(Fig. 49D)**. While total P levels in Pi-deficient seedlings reached the levels observed in Pi-sufficient conditions upon ATP supply, such gain was muted in the KO lines, indicating *SlPAP15* involvement in PAE in tomato **(Fig. 49E)**.

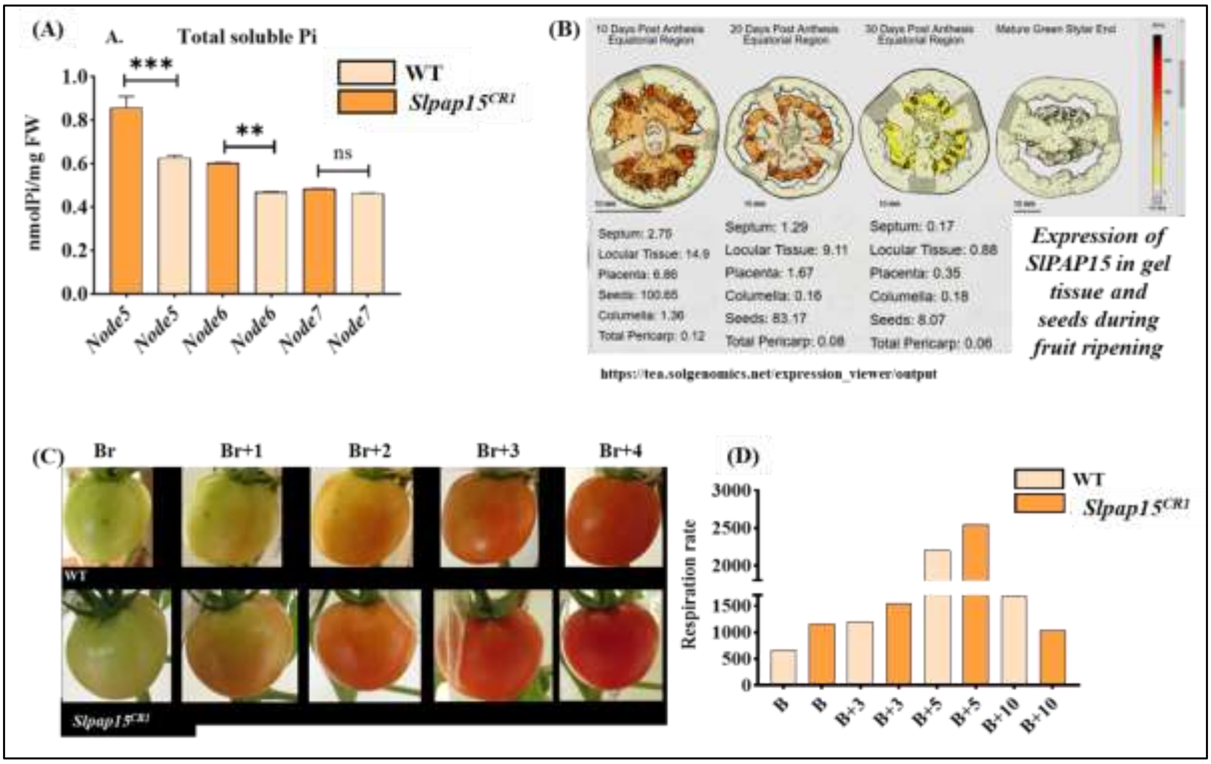


Fig 50: Preliminary results indicate a role of SlPAP15 in seed development and fruit ripening (A) *Total soluble Pi content in wild type (WT) and Slpap15*^{CR1} *KO lines grown in the greenhouse at* T_2 *generation* ***(B)*** *Expression of SlPAP15 in M82 background fruits in developing seeds and gel tissue* ***(C)*** *Fruit ripening progression in WT and the KO lines* ***(D)*** *Respiration rate in the WT and Slpap15*^{CR1} *line fruits during early ripening stages.*

We also noticed the lower Pi content in the leaves of different nodes in the KO lines growing in the greenhouse at T_2 generation. This explains its expected role in maintaining Pi uptake and homeostasis throughout plant development. The significantly affected seed germination in the KO lines is also intriguing, as *SlPAP15* transcripts are barely detectable in the pericarp tissues of fruits in multiple genetic backgrounds. Interestingly, in RNA-seq data available for the M82 background of tomato, *SlPAP15* transcripts are present in seeds and gel tissue **(Fig. 50B)**. Given the reduced phytase activity and lower Pi levels throughout the development, we anticipate a prominent role of SlPAP15 in mobilizing Pi from organic-P pools in the fruit gel tissue to the developing seeds at early fruit developmental stages **(Fig. 48E, 50A)**. Therefore, we checked the fruit ripening trait in the *Slpap15*^{CR1} KO lines at T_2 generation **(Fig. 50C)**. Preliminary data suggest early ripening phenotype and higher respiratory rate in the fruits of KO lines compared to that of the wild type **(Fig. 50D)**.

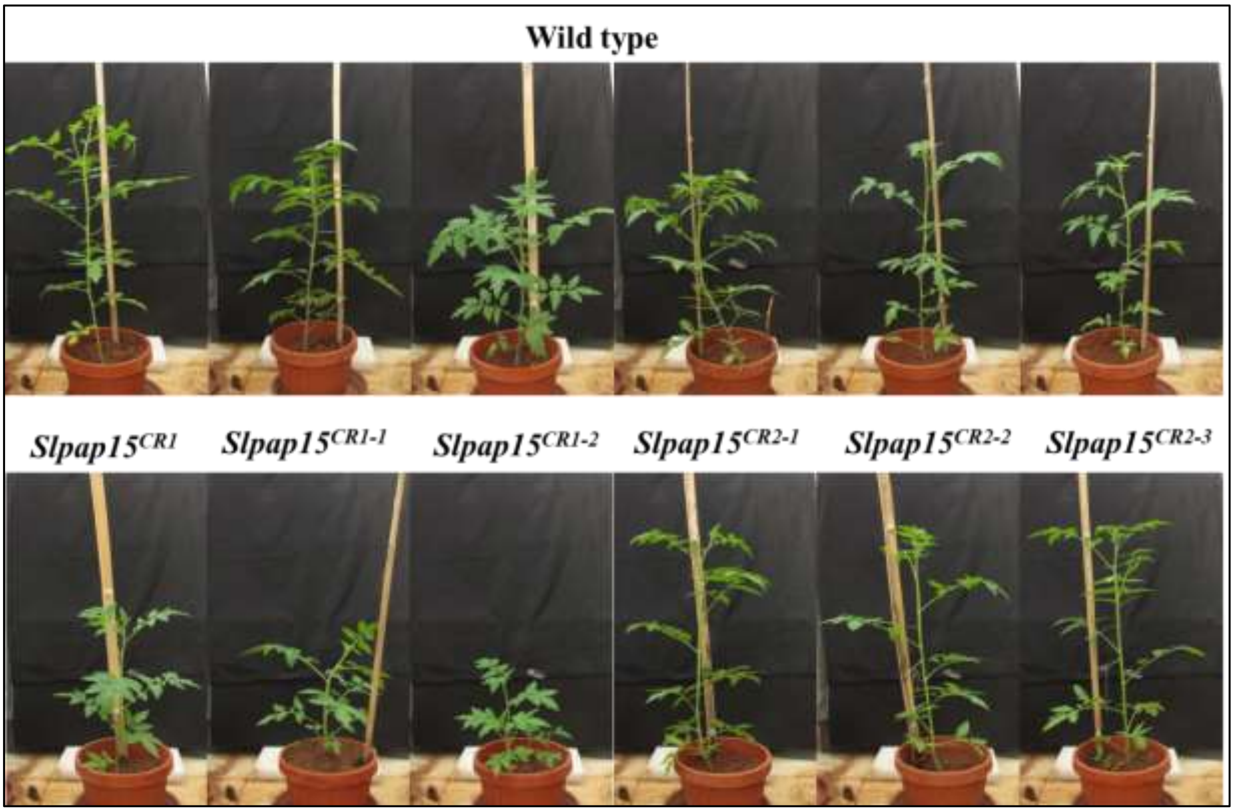


Fig 51: Three months old, wild type, Slpap15^{CR1} and Slpap15^{CR2} T$_2$ plants growing in the greenhouse.

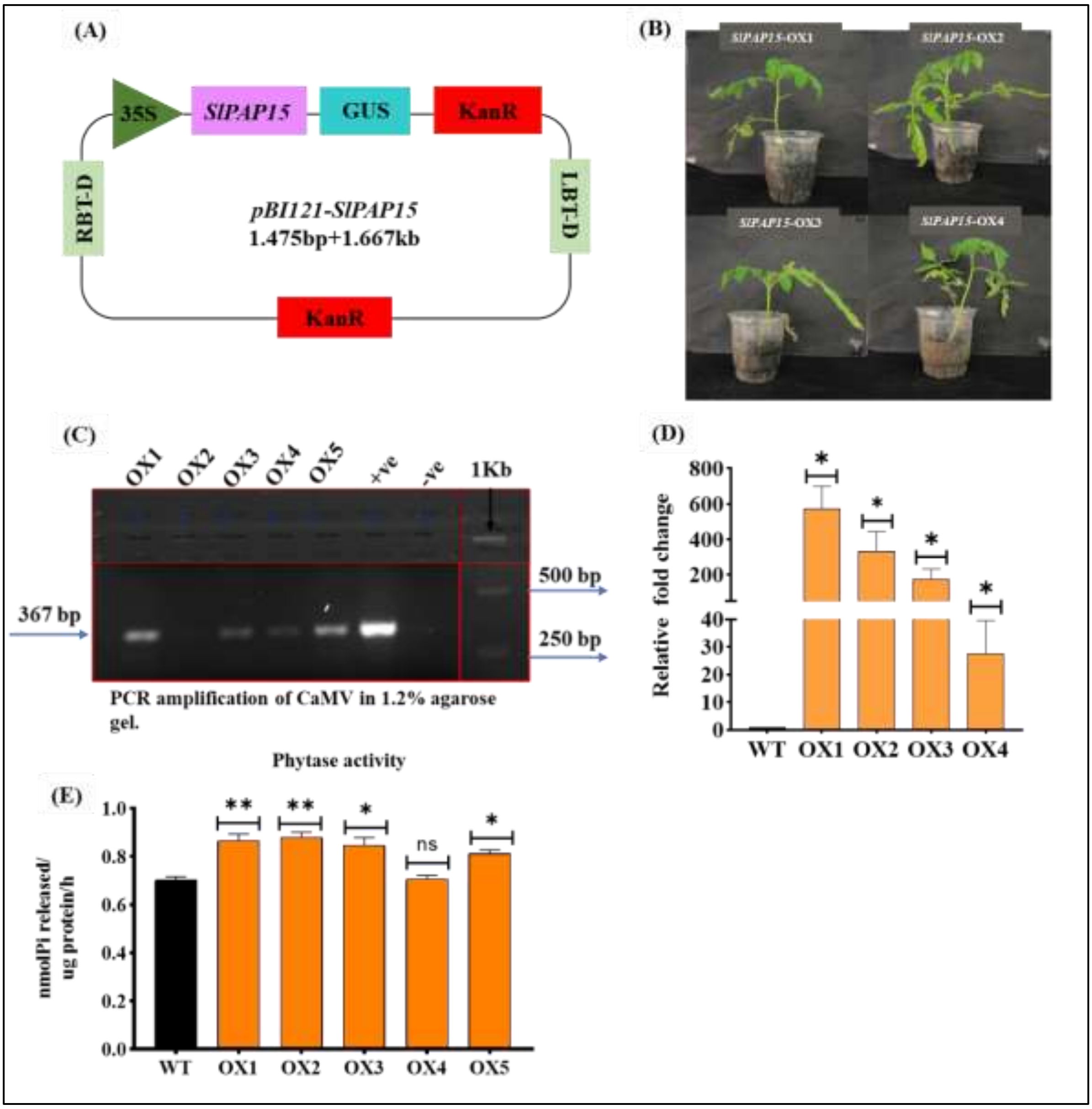


Fig 52: Generation of SlPAP15 stable overexpression tomato lines (A) *Vector map of pBI121-SlPAP15 used to generate the overexpression (OX) lines* ***(B)*** *Seedlings phenotype of OX lines in culture room.* ***(C)*** *PCR amplification of CaMV35S promoter fragment using promoter-specific primers for confirmation of SlPAP15-OX lines* ***(D)*** *RT-qPCR based confirmation of the SlPAP15-OX (1-5) lines* ***(E)*** *Phytase activity in the 4th node leaves of OX plants and wild type in two-month-old plant growing in greenhouse.*

2.9.8. Generation of *SlPAP15*-OX lines and their functional characterization

Next, we generated *SlPAP15* stable overexpression lines using the pBI121 expression vector. In total, 15 independent *35S:SlPAP15* OX lines were confirmed at the molecular level using *CaMV35S*-F and gene specific Rev primers. Of these, 5 OX lines were finally confirmed using RT-qPCR for the level of *SlPAP15* upregulation **(Fig. 52C, D)**. A very high transcripts level of *SlPAP15* was observed in all five OX lines than wild type. Phytase activity was checked in the leaves of two months old OX lines and wild-type growing in the greenhouse. Phytase activity was found to be enhanced in all OX lines compared to the wild type **(Fig. 52E)**.

Objective 3:

3. Functional characterization of an arbuscular mycorrhiza-specific purple acid phosphatase gene for its role in AM colonization and tomato seedlings growth

Arbuscular mycorrhizal (AM) fungi are known to colonize the root systems of most land plants. AMF colonization promotes plant growth by enhancing nutrient availability to the host. AMFs also help assimilate Pi from the rhizosphere [279]. Several *Glomus* (new name *Rhizophagus*) species of the phylum Glomeromycota establish symbiotic associations with many plants, including crop species. Several mycorrhiza-specific plant Pi transporters, which bypass the roots Pi uptake and directly deliver Pi to the cortical root cells, have been identified and described in various plants [242,280–283]. In this objective, we identified AM-responsive PAPs (*SlPAP1*, *SlPAP9b*, *SlPAP18a*, and *SlPAP20*) and characterized *SlPAP20* for its role in mycorrhization using the VIGS approach. This gene was chosen due to its root-preferential transcript levels, non-PSI nature, and strong induction upon mycorrhization.

3.1. Optimization of the mycorrhizae inoculum and soil phosphate levels for effective mycorrhization in tomato

We first mined the earlier published transcriptome data to identify candidate AM-responsive genes in roots colonized with *R. irregularis*. Higher expression of target AM-responsive genes and novel non-PSI SlPAPs (*SlPAP9b*, *SlPAP20* and *SlPAP27a*) was noticed **(Fig. 53C)**. First, we optimized the mycorrhizae spores at LP condition i.e., 50 µM using a perlite based solid growth medium. Plants were first grown for 13 days in ½X Hoagland media containing 1.25 mM Pi. After this, plants were transferred to sand containing varied amounts of mycorrhizae. Then, parameters such as dry weight, total phosphorus content, total anthocyanin, and total soluble Pi content were compared among the treatments, and the best optimum conditions were used in further experiments. Plant growth performance was better phenotypically under LP condition when 5g mycorrhizae was used. Higher root length and better shoot phenotype was observed under LP condition with 5g mycorrhizae **(Fig. 53A, B)**.

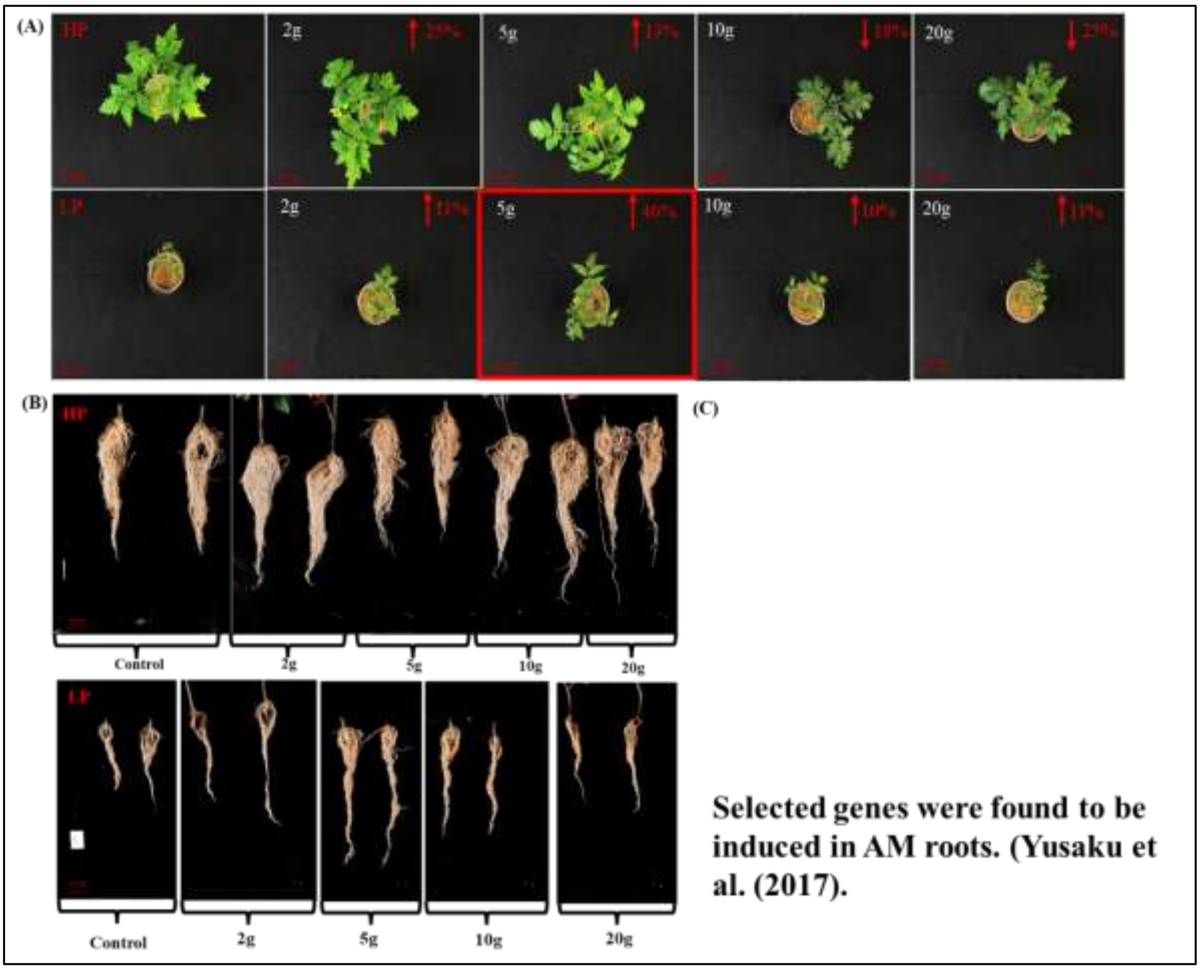


Fig 53: Optimization of mycorrhizae spores and Pi deficiency to study AM-responsive changes in plants. ***(A-B)*** *Shoot, and root phenotypes were observed in plants treated with a varied amount of mycorrhizae inoculum and Pi levels (HP, 1.25 mM and LP 50 µM).* ***(C)*** *Expression profile of PAPs and PTs in tomato root transcriptome data published by Yusaku et al. (2017).*

We found a gradual decrease in dry weight after 5g concentration at HP and LP conditions **(Fig. 54A, B)**, while total phosphorus content increased gradually under LP condition, but no change was noticed under HP conditions **(Fig. 54C, D)**. Anthocyanin accumulation, a major plant stress response under Pi starvation, was measured in these plants. At 5g mycorrhizae, lower accumulation of anthocyanin content, irrespective of the phosphate concentration, was detected. However, anthocyanin content increased after the 5g concentration in the HP condition **(Fig. 54E, F)**. We also checked the change in total soluble Pi content, which decreased significantly at higher concentrations of mycorrhizae used in both treatments **(Fig. 54G, H)**. Parameters such as MGR (mycorrhizal growth response) and MER (mycorrhizal effectivity ratio) was found to be increased by 46 % and 59 %, respectively, in plants treated with 5g number of mycorrhizae under LP condition **(Fig. 54I, J)**. After this, we also optimized Pi concentration in LP conditions for the best MGR and MER.

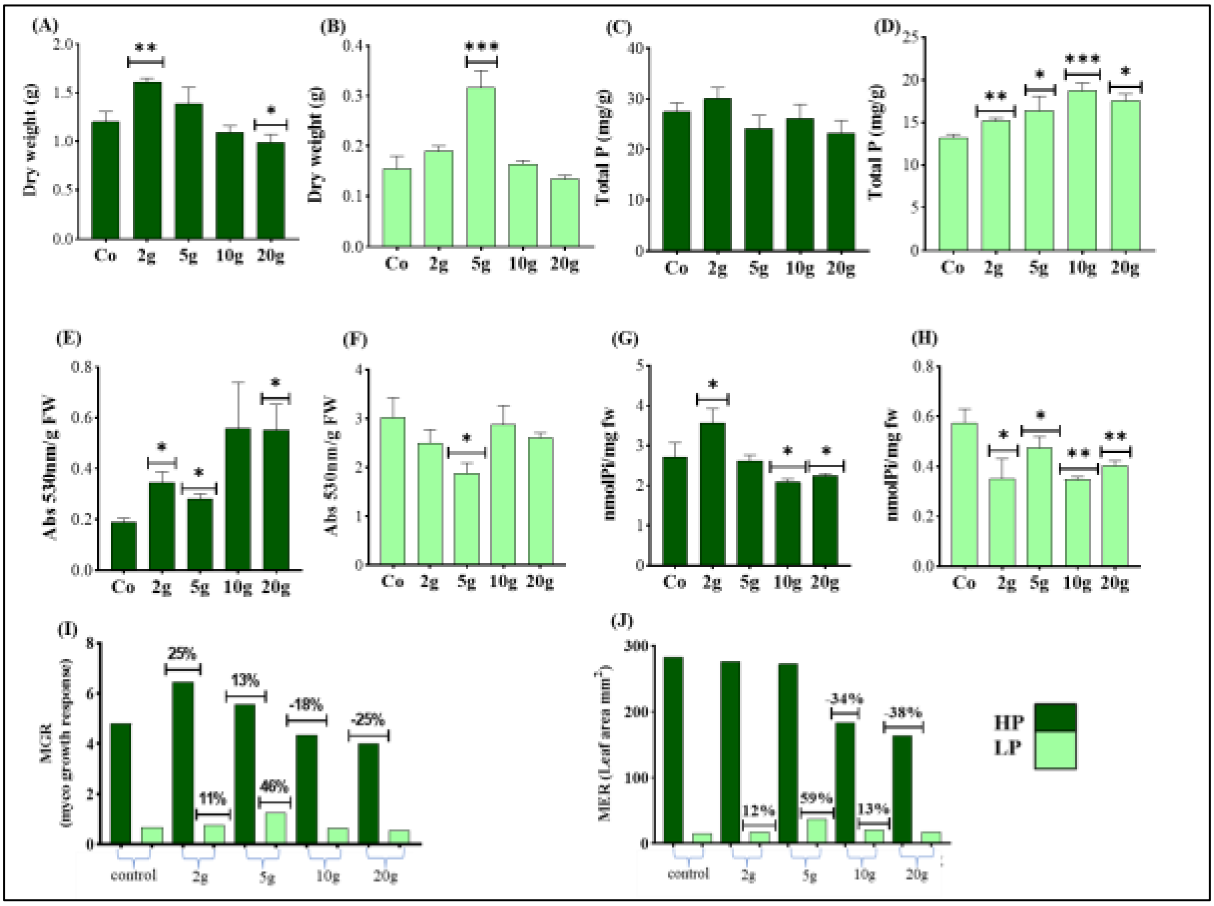


Fig 54: Optimization of AMF inoculum for optimum growth of tomato seedlings. (A-B) *Dry weight* ***(C-D)*** *Total phosphorus content at different mycorrhizae concentrations* ***(E-F)*** *Total anthocyanins content.* ***(G-H)*** *Total soluble Pi content* ***(I-J)*** *MGR (mycorrhizal growth response) and MER (mycorrhizae effectivity ratio) were measured upon colonization.* ***HP*** *= 1.25mM and LP = 200 µM.*

MGR was found to increase by 30 % at 50 µM, 14 % at 100 µM, 30 % at 200 µM and 13 % at 500 µM respectively. While MER increased by 32 % at 50 µM, 100 µM at 14 %, 200 µM at 31 % and 500 µM at 30 %. Surprisingly, both MGR and MER were high at 200 µM concentration compared to other concentrations **(Fig. 55C, D)**. Physiological and biochemical parameters such as dry weight (root and shoot), total biomass, total phosphorus, anthocyanin content and PAE were measured to reconfirm the above response. Shoot dry weight increased at 200 µM and 500 µM concentrations, while root dry weight increased significantly at 100 µM and 200 µM Pi concentrations upon colonization, which is also reflected at morphological level **(Fig. 55A, B)(Fig. 56A, B)**. The total biomass of seedlings increased at 100 µM, 200 µM and 500 µM Pi concentrations in the mycorrhizae experiments **(Fig. 56C)**. Total anthocyanin content decreased upon colonization at 100 µM and 200 µM concentrations **(Fig. 56D)**. We measured total phosphorus content to confirm whether phosphate has a role in the reduced stress response in tomato seedlings at 100 µM and 200 µM Pi concentrations.

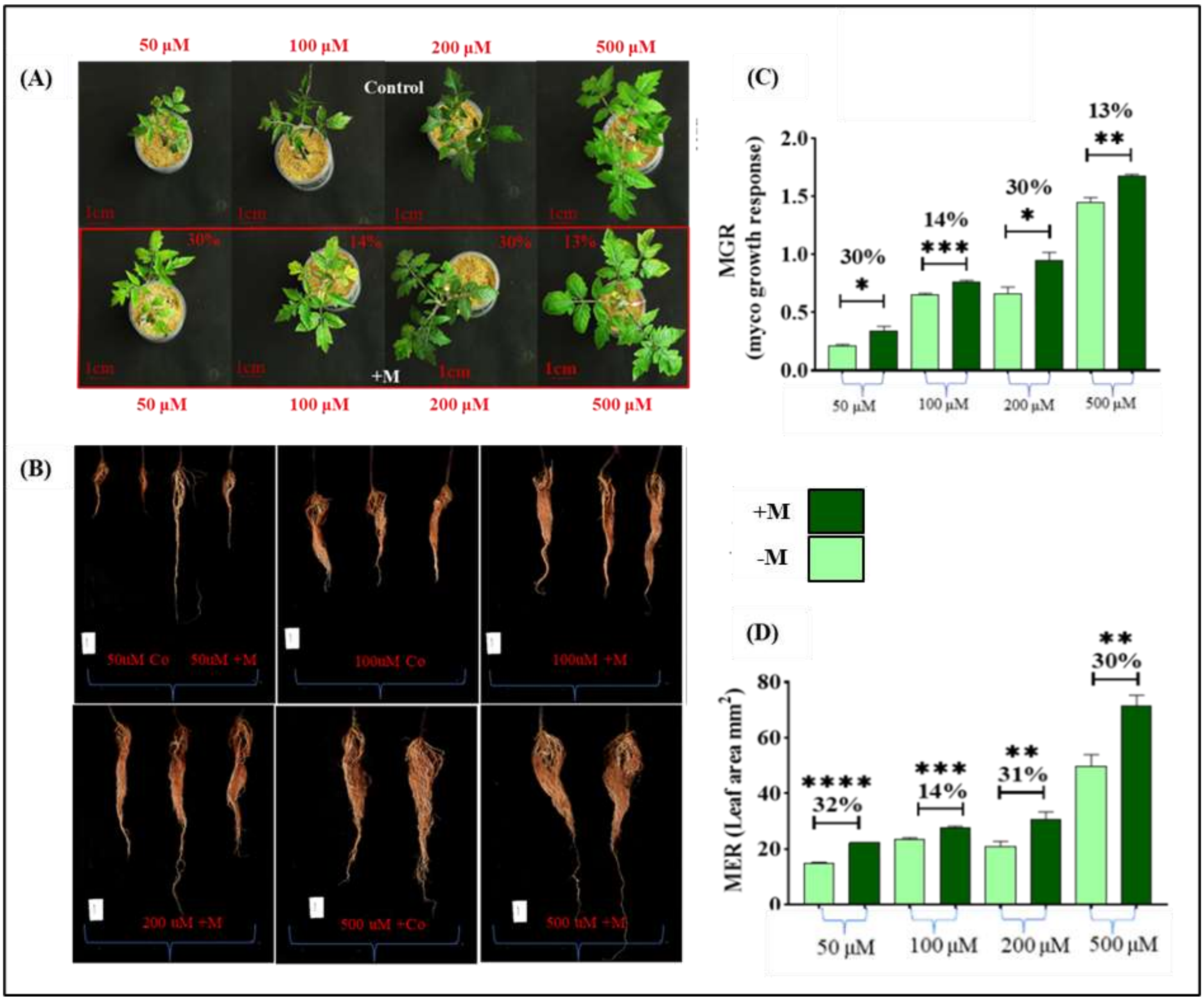


***Fig 55: Optimization of Pi concentration in mycorrhizae experiment. (A-B)** Shoot and root growth phenotypes upon mycorrhization at different P concentrations **(C-D)** MGR and MER for seedlings growth response. +M refers to mycorrhizae colonized seedlings, -M refers to uncolonized seedlings.*

Total phosphorus content increased significantly upon colonization at 200 µM and 500 µM Pi concentrations **(Fig. 52E)**. In line with high P levels, PAE increased significantly at all four different Pi concentrations used in the study **(Fig. 52F).** Altogether, the results showed a better growth response of plants grown at 200 µM Pi concentration with 5g mycorrhizae inoculum.

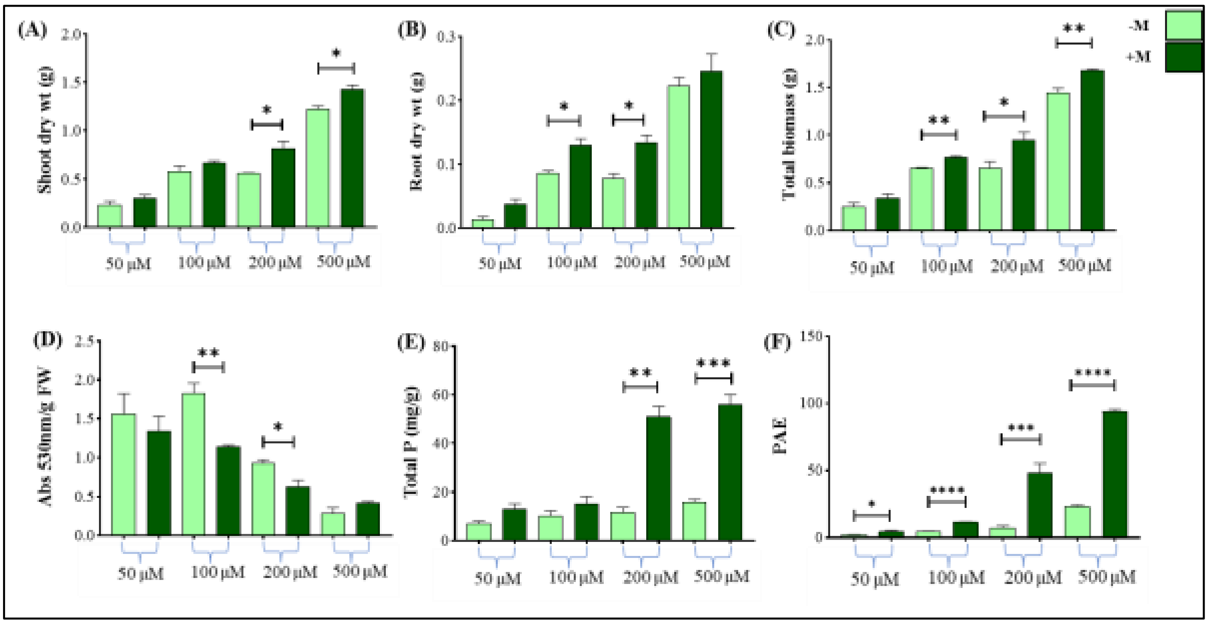
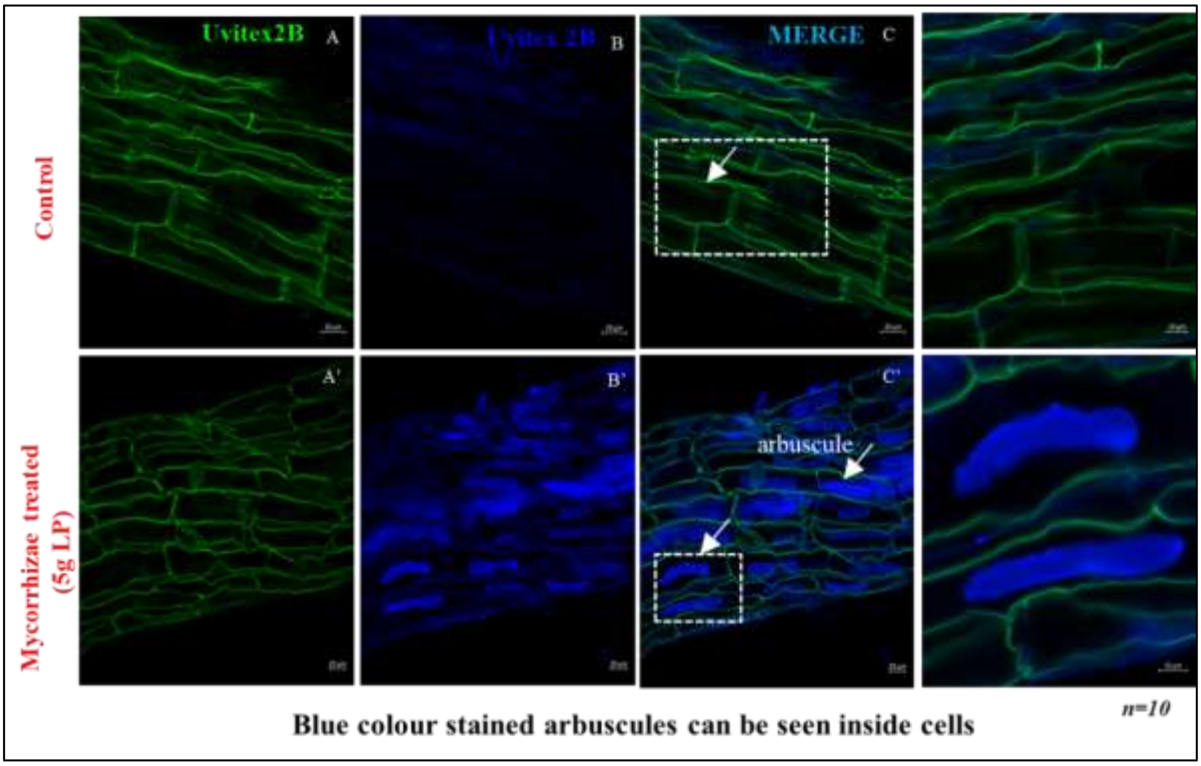


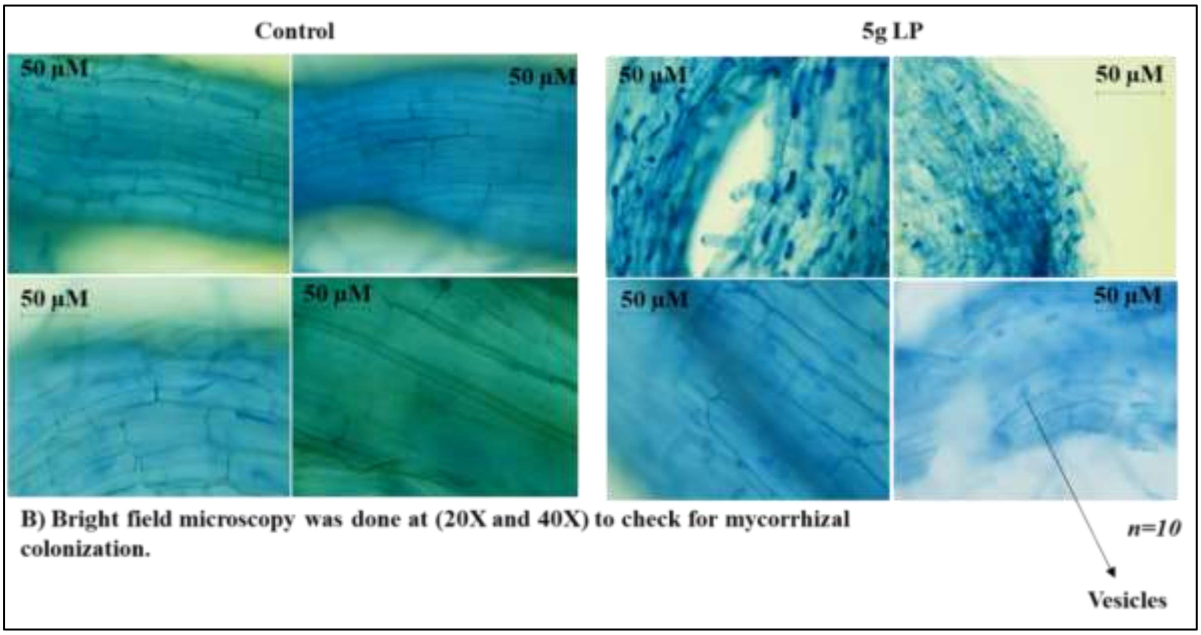
Fig 56: Biochemical analysis of AM colonized (5g) seedlings with different Pi concentrations (A) *Shoot dry weight* ***(B)*** *Root dry weight* ***(C)*** *Total biomass* ***(D)*** *Total anthocyanins content.* ***(E)*** *Total phosphorus content* ***(F)*** *PAE (Phosphorus acquisition efficiency). -M refers to un-colonized seedlings, and +M refers to the mycorrhizae colonized seedlings.*

3.2. Confirmation of mycorrhization in roots using Uvitex-2B and Pelikan blue dyes

To check for the mycorrhizal structures, such as arbuscules and vesicles in the AM-colonized roots, we performed staining of roots with Uvitex-2B dye, Pelikan Ink-blue stain, and trypan blue. For infestation of arbuscules, two independent acquisition was performed at one at 405/420-480nm (excitation/emission) for Uvitex2B and one at 488/530nm LP for autofluorescence, with pinhole sizes of 102 and 100 µM, respectively. We stained the roots with non-fluorescent dyes such as Pelikan blue to further reconfirm the colonization. Infestation of vesicles was observed inside the cells **(Fig. 57A, B)**.



Blue colour stained arbuscules can be seen inside cells



B) Bright field microscopy was done at (20X and 40X) to check for mycorrhizal colonization.

Fig 57: Histochemical staining of mycorrhizae colonized root using fluorescent and non-fluorescent dye (A) *Uvitex-2B staining of roots colonized with mycorrhizae.* ***(B)*** *Pelikan blue staining of roots to observe the fungal structures, such as arbuscules and vesicles inside the cells.*

3.3. Expression profiling of PAPs and AM-responsive genes

To further confirm AM colonization at the molecular level, we also checked the expression of already identified AM-responsive genes, from the transcriptome data, such as *SlPT4, SlGH3.4,* and *SlSYMRK*. Higher expression of *SlGH3.4* in roots was noticed at HP and LP conditions upon mycorrhization **(Fig. 58D)**. While *SlPT4* and *SlSYMRK* were upregulated upon mycorrhization only under LP conditions **(Fig. 58B, E)**. The higher expression of these genes correlates with the biochemical results, where we noticed high phosphorus content and improved mycorrhizal growth response at the optimized Pi concentration. After the biochemical, microscopic, and molecular confirmation, we performed further experiments with the optimized amount of P and mycorrhizae inoculum. Next, we checked the expression of SlPAPs genes in the AM-colonized tomato roots. Interestingly higher expression of *SlPAP1*, *SlPAP9b*, *SlPAP18a*, and *SlPAP20* was noticed, which correlates with the earlier published transcriptome data in roots by Yusaku et al. (2017) **(Fig. 59D, E)**[284]. *SlPT4* was used as a positive control in this experiment. Interestingly, except *SlPAP1*, the remaining three (*SlPAP9b*, *SlPAP18a* and *SlPAP20*) PAPs are the non-PSI genes. Expression profiling of these *SlPAPs* showed high expression of *SlPAP1* and *SlPAP20* in roots and unopened flower buds. Transcripts accumulation of *SlPAP9b* is more in unopened flower buds. *SlPAP20*, being a non-PSI and AM-responsive gene with its root-preferential expression, was selected for further functional characterization **(Fig. 59A-C)**.

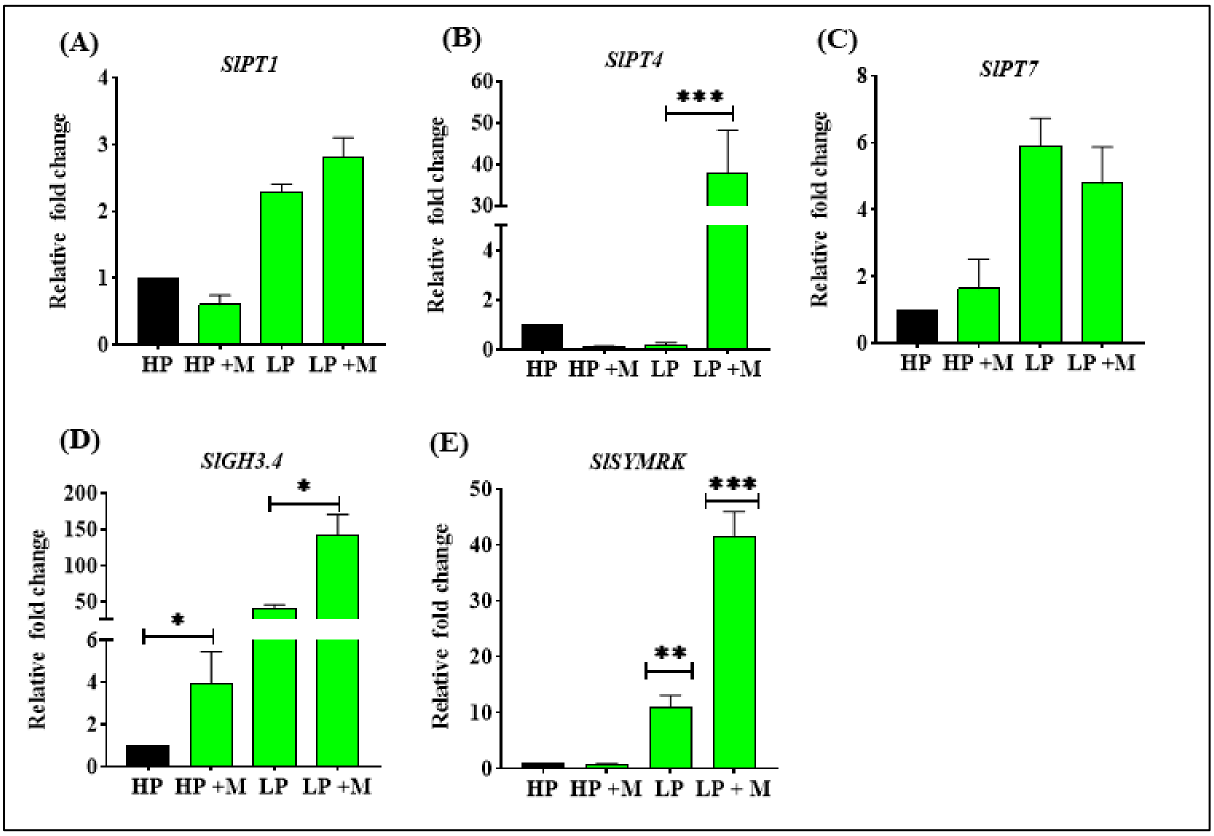


***Fig 58: Expression of AM responsive genes in optimized growth conditions at HP (1.25 mM) and LP conditions (200 µM). (A-C)** PTs, Pi transporters **(D)** Auxin metabolism gene SlGH3.4*

***(E)** SlSYMRK, a symbiotic receptor-like kinase. HP = 1.25 mM and LP = 200 µM. +M indicates mycorrhizae colonized roots.*

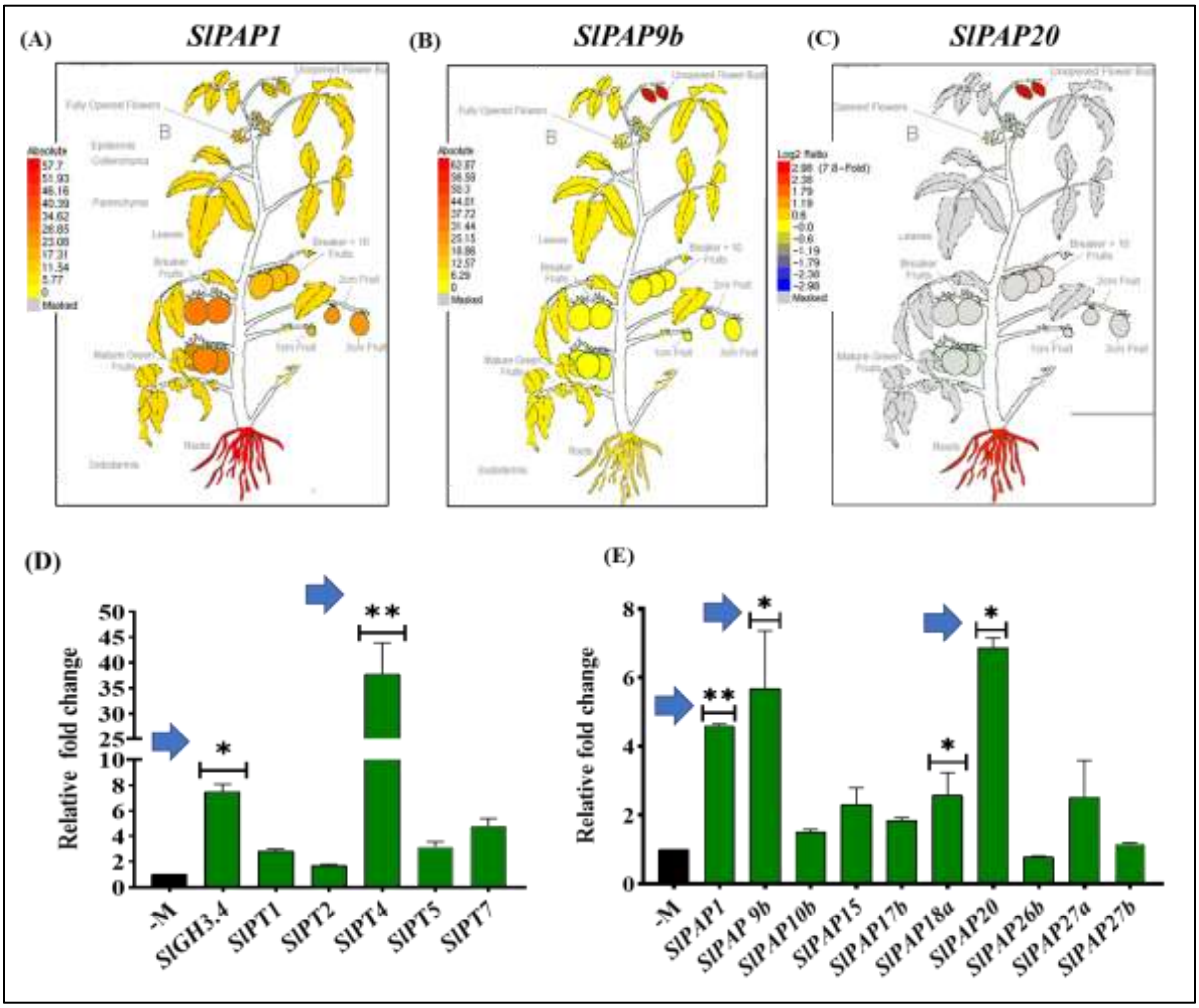


***Fig 59: Expression profiling of AM-responsive PAPs. (A-C)** Transcript profile of SlPAPs at different plant growth and development stages using multi-Plant eFP Browser 2.0 – BAR tool and RT-qPCR. **(D)** Relative expression of AM-specific responsive genes at the transcript level. **(E)** Relative expression of SlPAPs upon mycorrhization under LP condition (200 µM) four weeks post AM-colonization. -M is the control (non-colonized roots), for checking the expression of AM-responsive genes.*

3.4. Functional characterization of *SlPAP20* for its role in mycorrhization

While PAPs are well investigated for their roles in PSR, their role in AM-colonization is poorly understood. We characterized *SlPAP20* using VIGS for its role in AM colonized roots to get primary insights into their function. The silencing efficiency was confirmed using *SlPDS*, as mentioned earlier. We observed an excellent silencing efficiency of around 80 % of infiltrated plants with photobleached leaves. Further silencing confirmation of *SlPAP20* was also done using RT-qPCR in both -M and +M conditions **(Fig. 60C)**. Morpho-physio analysis confirmed the lower accumulation of total soluble Pi and total P content in the silencing plants. Interestingly total biomass and PUE were significantly increased in the silenced seedlings upon mycorrhization **(Fig. 61A-D)**. We did not observe any change in the non-colonized silenced seedlings **(Fig. 61E-H)**.

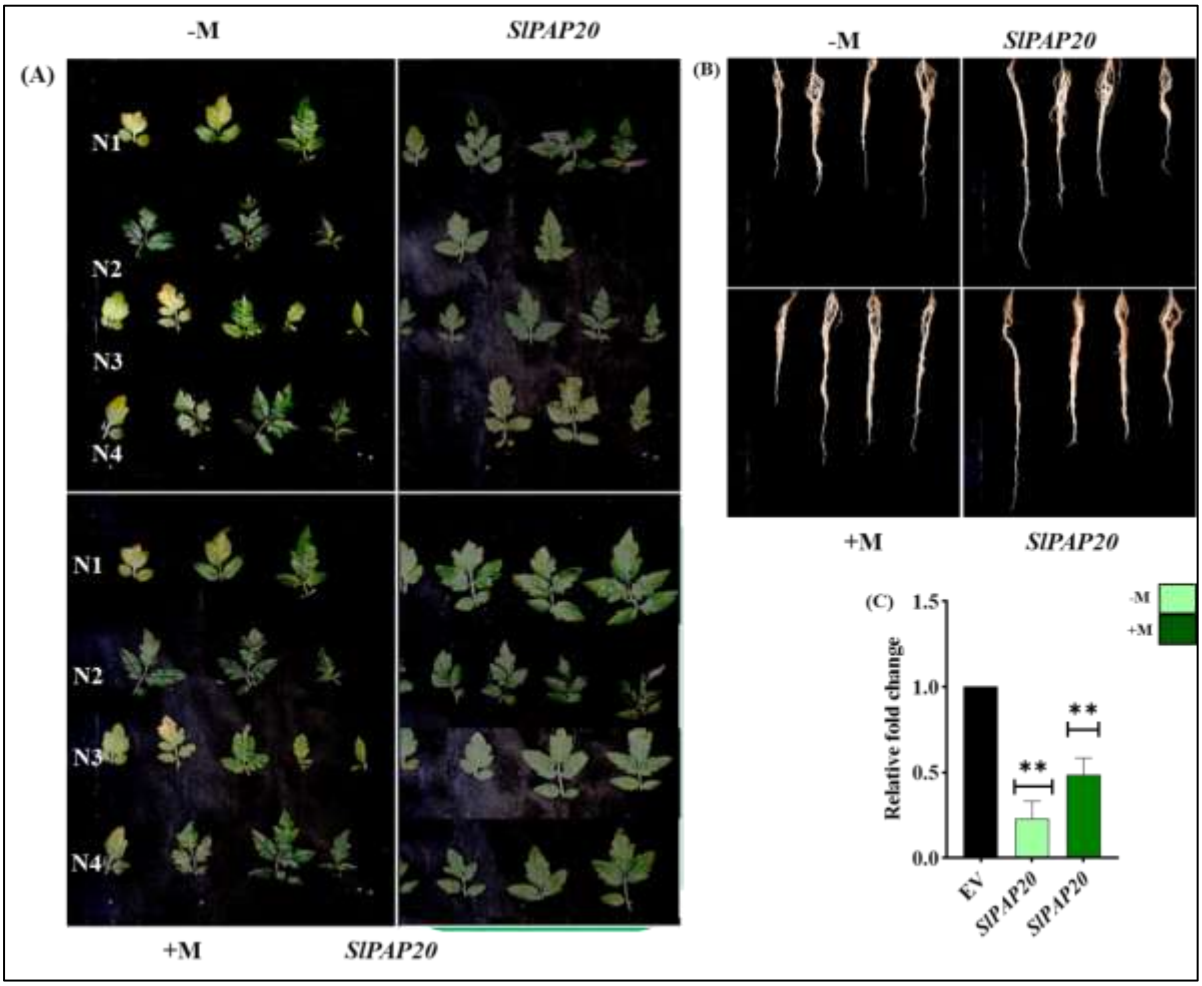


***Fig 60: Silencing of SlPAP20 resulted in better growth response upon mycorrhization in tomato seedlings under LP (200 µM) condition**. **(A)** The leaf area of both the silenced and unsilenced seedlings under +M (colonized) and -M (uncolonized) conditions **(B)** Primary root length **(C)** Confirmation of silencing at the molecular level using RT-qPCR.*

We also checked whether silencing of *SlPAP20* affected AM colonization and found a higher infestation of mycorrhizae in the silenced roots. This higher mycorrhization was also quantified by counting the number of vesicles infested in roots **(Fig. 62A, B)**.

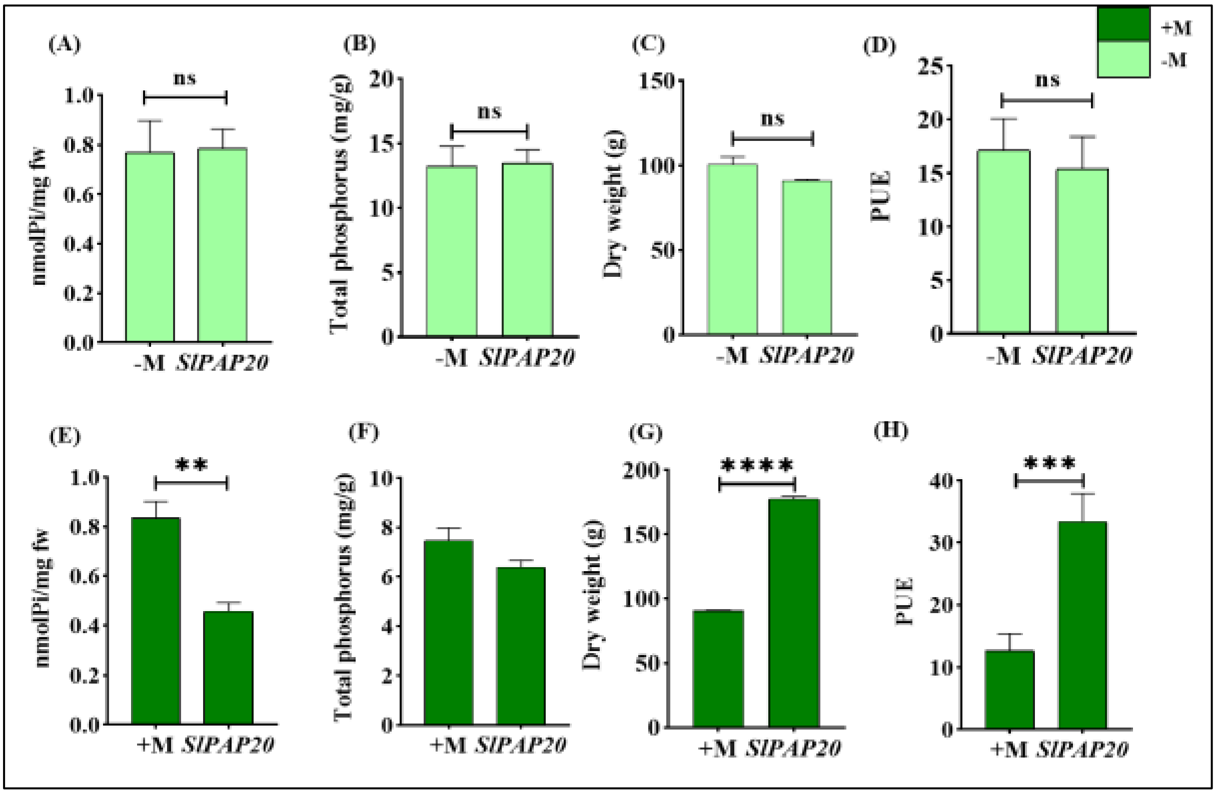


Fig 61: Morpho-physiological analysis of SlPAP20 silenced and non-silenced seedlings upon mycorrhization. (A-B) *Total soluble Pi content and total P in -M and SlPAP20 silenced seedlings* ***(C-D)*** *Dry weight and PUE in non-colonized and silenced seedlings* ***(E-F)*** *Total soluble Pi content and total P in +M and SlPAP20-silenced seedlings* ***(G-H)*** *Dry weight and PUE was measured upon mycorrhization in SlPAP20-silenced seedlings. -M and +M = non-colonized and colonized roots.*

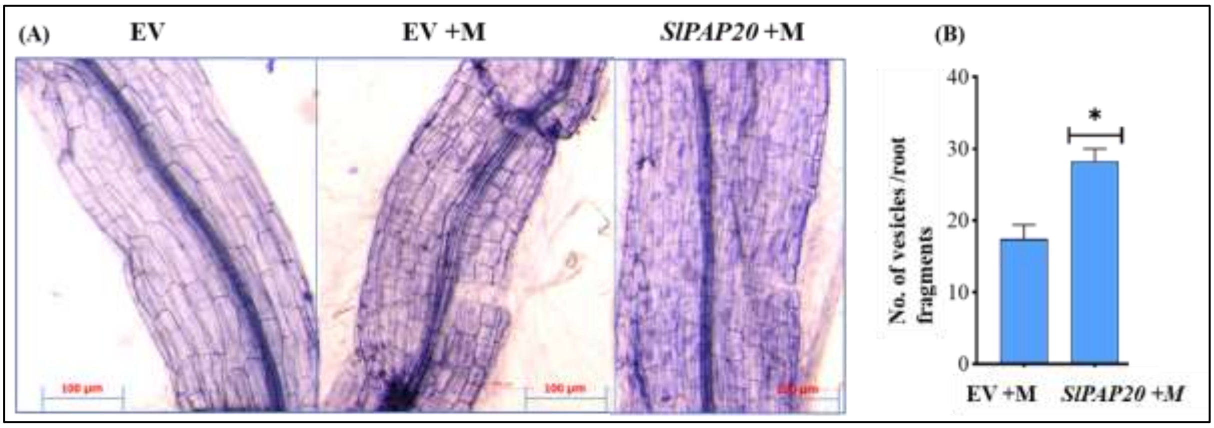


Fig 62: Histochemical staining of SlPAP20 silenced roots. (A) *Pelikan blue staining of control and silenced seedlings to observe fungal structures in roots* ***(B)*** *No. of vesicles were counted in root fragments colonized with mycorrhizae. N= 5, n=10 (N= Number of roots, n= Number of root fragments from each root). The experiment was repeated at least twice.*

Discussion

Tomato cultivation encompasses a range of varieties, each exhibiting unique reactions to environmental stresses. The study aims to identify and evaluate the tolerance and sensitivity of tomato germplasm under P deficient condition. Tolerant varieties are expected to behave as resilient and adaptable genotypes compared to the sensitive one. While sensitive varieties can be more susceptible to damage and yield loss when exposed to P deficiency [285]. Hence, the selection and cultivation of tolerant varieties are important to ensure stable yield and adaptability to phosphate deficiency. In this study, based on the morpho-physiological parameters and certain biochemical assays, screening of limited tomato germplasm at 4-week-old seedlings stage was performed. Based on their performance under P starved condition, candidate tolerant and sensitive genotypes were identified and selected and studied in depth using the omics approach.

Concentration of bioavailable P form i.e., inorganic phosphate (Pi), in soil is generally low is (~2 μM), and is far less than that is maintained inside plants (generally in mM range)[8]. Plants employ an array of well-coordinated morphological, physiological, biochemical, and molecular adaptations to orchestrate a varied response under chronic Pi limitation [90]. The comprehensive morpho-physiological analysis revealed a more substantial effect of Pi starvation in sensitive genotypes which leads to higher anthocyanin content, lesser biomass and PUE [286]. Recent studies on screening of different plant species also reported that plant photosynthetic efficiency is compromised in the sensitive genotypes, which is directly linked to sugar metabolism [287]. In our study, we also anticipated the reduced photosynthetic efficiency in sensitive tomato genotypes. This was confirmed by our physiological data during screening where lower Fv/Fm ratio and PSII is recorded in the sensitive accessions. This in turn can affect sugar metabolism or biosynthesis inside plant. For example, in Glycine max, trehalose content and starch content increase with the improved performance index of PSII of drought tolerant cultivar, UVE14 [288]. The increased number of transcripts of bidirectional sugar transporters in tolerant genotypes further hints, that internal sugar metabolism may be responsible for the observed better growth response in the tolerant genotypes. We also witnessed the increased number of root hairs and lateral root density, which is a more robust response by plants under Pi starvation, in the sensitive varieties. The same is also observed in many species such as in *Arabidopsis thaliana*, *Phaseolus vulgaris*, *Oryza sativa* and *Fraxinus mandschuria* [289–293]. The change in RSA under phosphate deprivation is also associated with release of secretory

APase, ribonucleases and also enhanced ROS production [294]. The prominent class among APases is generally purple acid phosphatases (PAPs), which are either secretory or intracellular in terms of their functions [295]. In our study plant secretory acid phosphatase activity and root surface associated APase activity were found to be less perturbed in tolerant genotypes. In search of the expected PAPs, responsible for enhanced activity in the sensitive accessions, we first identified the full complement of 25 PAPs in the tomato genome [52]. We also identified PSI SlPAPs using RNA-sequencing and RT-qPCR analysis. We next performed RT-qPCR analysis of candidate root-preferential PSI SlPAPs in tolerant and sensitive varieties and found that the levels of their induction under Pi starvation in the two sets of genotypes correlates with the earlier published results and the observed SAP activity [296,297]. RNA sequencing is a useful tool for profiling and quantifying transcripts in tomato. The activation of more transcripts in tolerant genotype (716) than in sensitive (315), in our RNA-seq data of Pi starvation seedlings, is surprising but reflects the tolerant nature of genotypes which also correlates with the earlier observations reported by, Suresh et al. (2021)[296]. In the RNA-seq data, the authors have shown higher number of transcripts detected in the tolerant rice genotype (NIL-23) compared to that in sensitive (Pusa-44). However, a decrease in number of downregulated genes (413) in tolerant genotype compared to that in the sensitive variety (488) further supports the findings. Altogether, activating a larger set of genes than downregulated ones in tolerant seedlings reiterates the significance of gene activation over gene suppression in PSR. The presence of 95 commonly expressed genes and 65 downregulated genes implicates a broader role of these genes in Pi homeostasis across accessions in tomato. Number of components including plasma membrane, organelle membrane and other cellular compartments, which are associated with integral component of membrane, and metabolic pathways related to glycolytic and lipid metabolic process are expressed more in the tolerant genotype. Concerning the ‘molecular function’ in which they are involved in terms of their metal binding site, heme binding and iron-ion binding are more involved in tolerant genotypes. Reprogramming of root system architecture (RSA), generally to increase the surface area of roots, is a central response to almost all nutrient deficiencies [298]. Production of reactive oxygen (ROS) such as superoxide anion (O^{2-}) and hydrogen peroxide (H_2O_2) invariably underlie such changes in RSA. Interestingly, we found transcripts of genes related to peroxidase, monooxygenase, oxidoreductase and hydrogen peroxide catabolic process are detected more in sensitive genotypes, which is in correlation with high ROS accumulation in them. Transcripts of SlSPXs are also expressed more in number in sensitive genotypes than tolerant genotypes, while no such change was noticed in the expression of putative SlPHRs. Sharma et al. (2020) reported the identification of 127 MYB genes in the tomato genome [299]. Tolerant varieties exhibit differential expression of MYB transcription factor genes such as *SlMYB41*, *SlMYB75*, *SlMYB61* and *SlMYB113*. Differential expression of transcription factor families like, MYB, WRKY and AP2 like in our transcriptome is in line with the earlier published transcriptome with contrasting genotypes of rice [296,300].

Production and secretion of acid phosphatases (APase) is a characteristic adaptive strategy by plants to respond to Pi deprived conditions. PAPs are APases with broad substrate specificity and play a pivotal role in maintaining P homeostasis in plants by hydrolysing various organic P-compounds in acidic pH [57,245,301–303]. Maintenance of intracellular P homeostasis is controlled by highly interconnected genetic mechanisms among which the pivotal role of PAP gene family members have been emphasized in many studies [74,304–306]. PAP gene family has been identified in many plant species with variation in the number of members [307,308] . PAPs are induced upon Pi deficiency, although to the varied number in different species [72,309–311]. Up-regulation of many acid phosphatases in Pi-starved seedlings in this study suggests a broad set of phosphatases are involved in tomato PSR. This is also reflected by the increased intracellular (IAP) and secretory (SAP) APase activity in the LP grown tomato seedlings, especially under prolonged treatment [295,312]. However, it is in contrast to the activation of only one PAP gene (Gm03g00380) in soybean[313]**.** Previous studies have implicated four purple acid phosphatase genes in the regulation of enhanced PAP activity in tomato [295,312]. Data mining revealed existence of 25 SlPAP members each in cultivated tomato and its wild relative *S. pennellii* genomes; a significant increase in their number over previous reports [295,312]. This outcome is also in accordance with the previous such genome-wide identification studies, where similar number of PAP genes have been identified in chickpea and *Jatropha curcas*, suggesting noteworthy conservation in their number in many plants, irrespective of the size of their genomes [57][64]. Phylogenetic analysis revealed that similar to Arabidopsis homologs, tomato SlPAPs form three main groups (I, II, III) and seven subgroups (Ia-2, Ib-1, Ib-2, IIa, IIb, IIIa and IIIb [314]. Protein sequence of tomato PAPs are more similar to SpePAPs and AtPAPs than their rice homologs suggesting higher probability of their conserved functions and behaviour in dicots than in monocots. Absence of several AtPAP direct homologs, including AtPAP5, AtPAPA6, AtPAP11, AtPAP14, AtPAP16, AtPAP19 and AtPAP28 in tomato and S. pennellii genomes is in conformity with earlier reports, such as in chickpea, suggesting contribution of species-specific loss and/or divergence in the evolution of these genes in plants [57]. It further suggests that several tomato PAPs, for which no direct homologs could be identified in Arabidopsis, might have diverged for special functions, and demands further investigations for deciphering such roles in the Solanum genus. For instance, the pronounced transcript levels of SlPAP15, SlPAP26b and SlPAP27b homologs in roots, SlPAP17a and SlPAP27c homologs in flower, and SlPAP9a and SlPAP24b homologs during fruit ripening in more than one genetic background suggests their involvement in maintaining P-homeostasis in the respective tissues/stages [245,315]. No strict correlation between gene expression and phylogenetic analysis is in accordance with the earlier observations in Jatropha and maize, suggesting that functional divergence at the level of gene expression may occur rapidly in SlPAPs during evolution [64,316] Considering that the previously reported isoforms are the ones predicted here, it will also be interesting to discern, if SlPAP15 interacts with any one of the two SlPAP26 isoforms and form a heterodimeric complex, as shown by Bozzo et al. (2006)[295]. Earlier, *AtPAP10, AtPAP15* and *AtPAP26* have been characterized as root-surface associated intracellular and secretory SlPAPs [65,245,315]. In the present study, the strong activation of their tomato homologs after 15 days of Pi deprivation, predominantly in the root tissues. Presence of metallophos specific binding domains in all tomato SlPAP members, barring SlPAP23a, is in congruence with their rice and Jatropha homologs. Two PAPs, OsPAP21c and OsPAP27c in rice and three PAPs, JcrPap20b, JcrPap27a and JcrPap29b in Jatropha have been reported to lack any metallophos specific binding [64,317] Evidence suggests that secretion of several SlPAPs depends on structural features of these proteins such as signal peptides, N-glycosylation sites and N-terminal transmembrane helix [53,318]. The presence of signal peptides and N-glycosylation sites in majority of tomato SlPAP members is in keeping with the earlier published results [64]. Nevertheless, a clear disagreement between the number of SlPAPs harbouring signal peptides, N-terminal transmembrane helix and GPI anchor sites in tomato and Jatropha members suggests towards functional divergence among the SlPAP members in the two [64,319]. Transcriptome study of 8D and 15D old seedling resulted in the higher expression of majority of SlPAPs at one of the time points in whole seedling. Validation of these SlPAPs using RT-qPCR of 8D and 15D old post starvation seedlings resulted in the activation of majority of tomato SlPAP genes under Pi deficiency [64,65,320,321]. This is further confirmed at protein level, where higher abundance level of several PSI SlPAPs were detected which are in correlation with that of our in-house transcriptome data. Beside this, we also checked whether these SlPAPs are induced by other mineral nutrients (-P, -Mg, -N, -K, and -Ca) deficiency. Except for *SlPAP7*, all the other SlPAPs expression are P starvation specific which reaffirms their restricted roles to PSR in plants. Literature also suggest higher levels of several sugars metabolites acts as an essential component required for the activation of PSI genes under low Pi conditions [322][98]. Based on their Pi-specific nature, higher expression and varied expression profile, we selected *SlPAP15*, *SlPAP17b* and *SlPAP26b* for detailed characterization. For functional characterization of the selected SlPAP genes, VIGS fragments were cloned in the pTRV2 vector for elucidating its role in PSR. The photo-bleached phenotype of leaves in the EV-*SlPDS* silenced VIGS plants confirmed the effectiveness of the VIGS experiments. As expected, contrary to their EV control (SlPAPs non-silenced), VIGS plants, the EV-*SlPAP15*, EV-*SlPAP17b* and EV-*SlPAP26b*, showed strong suppression of these genes. SlPAP17 has two paralogs, SlPAP17a and SlPAP17b, both of them belong to low molecular weight category (LMWs). In-silico analysis have predicted them to be of cytoplasmic in nature. While its close homolog in Arabidopsis, AtPAP17 is also a LMWs PAP, it is dual localized to the cell vacuole and extracellular matrix [323]. Higher sequence identity of SlPAP17b with AtPAP17 at nucleotide level indicates the genes might have conserved functions. We also found this gene to be sucrose dependent as evident by its enhanced transcripts level upon exogenous sucrose supply under low Pi condition. Because of its PSI and early responsive nature, as evident from time-course experiment, an active role of this gene in Pi homeostasis was anticipated. Strangely, the silencing of *SlPAP17b* did not affect the plant growth under HP and LP conditions. Although this observation is in line with the results in Arabidopsis where no phenotypic change in *atpap17* mutant seedlings were reported under LP and HP conditions [324]. However, we found lower PUE and significantly reduced total soluble Pi content in silenced tomato seedlings irrespective of the Pi regime. This is in contrary to the reported observations in soybean where *GmPAP17*-overexpression line showed better PUE [325]. The silencing of this gene did not affect plant overall growth, which could be due to the induction of other PAPs. In *atpap26* and *atpap17b* mutant plants, induction of *AtPAP17b* and *AtPAP26*, respectively, has been reported [304]. Contrary to the presence of single PAP26 member in Arabidopsis and rice, the identification of two SlPAP26 members in tomato is interesting and suggests that gene-duplication might have contributed to the evolution an additional member for some new functions. Further, highly distinct transcript profiles of SlPAP26a and SlPAP26b during vegetative and reproductive developmental stages/tissues suggests diverged functions for these two genes. Role of *AtPAP26* in delayed senescence and remobilization is well elucidated [326]. The strong ubiquitous upregulation of *SlPAP26b* at both time points in the present study and also in earlier published report by Pfaff et al. (2020), but not SlPAP26a, suggested it to be a PSI gene[277]. The expression pattern also indicates a predominant role of this gene over *SlPAP26a* in maintaining P homeostasis in tomato seedlings. The time-course gene expression kinetics of its activation and recovery indicated a stronger and more rapid changes in

SlPAP26b transcripts in shoot than root tissues under Pi-starvation. Transient silencing of *SlPAP26b* resulted in compromised seedlings growth under LP conditions, which is further supported by increased anthocyanin content and root length in the silenced plants. The decreased Pi and total P content in the *SlPAP26b*-silenced plants under LP conditions than their EV controls suggest its role either in mobilization or P acquisition. The results in our study are in line with the published findings on *atpap26* mutants under LP conditions [326,327]. Higher anthocyanins accumulation in the silenced plants explained the severity of P stress sensed in these plants under both HP and LP conditions. As anticipated, VIGS based characterization of both paralogs confirmed the more prominent role of *SlPAP26b* than *SlPAP26a* in regulating Pi starvation response in tomato seedlings. Due to the significant response obtained in the silenced seedlings, we were interested in investigating whether *SlPAP26b* is directly regulated by SlSPX-SlPHR regulatory module. The unchanged transcript levels of *SlPAP26b* in SlSPX1/SlSPX2-silenced and SlPHR1/SlPHL1-silenced plants indicates that its regulation is independent of at least these two SlSPX and SlPHR members. The down-regulation of *SlPT1* and *SlPT7*, the known direct targets of PHR1, in *SlPHR1/SlPHL1* silenced plants acted as the positive control in the experiment. Although the SlPAP26b promoter harbors a P1BS element (the site recognized by PHR1), no GUS activity of pSlPAP26b::GUS in the transient transactivation experiment in N. benthamiana indicates it to be independent of SlPHR1 and its homologs. The absence of P1BS element in the promoter region of rice PAP26b supports these results and our hypothesis of its SlPHR1/L1 independent regulation under Pi deficiency [65]. Such non-PHR1 independent induction of SlPAP26b transcripts was also validated by the induction of this gene in many other mineral nutrients deficiencies. It also implies the involvement of other cis-elements, such as W-Box (TTGACY), TC element (TCTCTCT) or NIT-like elements (AAATATCT) [328]. Next, we investigated the role of SlPAP15 in PSR. Wang et al. (2019) have reported that the overexpression of AtPAP15 in soybean can lead to improved growth response or Pi acquisition with phytate as the sole source of P [329]. Higher expression of this gene in root and flower bud is further confirmed at RT-qPCR level at different tissues. Similar to earlier characterized SlPAPs, *SlPAP15* is also a PSI gene which is strongly induced upon sucrose treatment under low Pi conditions. High sequence similarity of this gene with its homologs at protein level hints towards the conserved functions among different species. Higher expression of *SlPAP15* in SlSPX1/2 silenced seedlings and its lower expression in SlPHR1/L1 silenced seedling, indicates it to be directly regulated by SlSPX-SlPHR regulatory module. Hence, we also checked the activation of SlPAP15 promoter by SlPHR1/L1 in tobacco in an in-planta transactivation assay. We found SlPAP15 promoter is directly activated (through GUS accumulation) by both SlPHR1 and SlPHL1. Earlier reports have suggested the predicted role of SlPAPs associated with phytase activity should have the presence of all four motifs [330]. Out of the 25 earlier identified SlPAPs, the presence of all the five motifs were detected only in SlPAP15. Further, occurrence of the highly similar binding-residues of active cavities for the most preferred ligands such as Fe, NAG, Zn, and Co in tomato SlPAPs with 3-5 active pocket sites further indicates that while biological function of the close SlPAP homologs in different plant species may be conserved, reliance of their activity on multiple ligands remains promiscuous [64]. Highly significant conservation in the residues defining substrate specificity among tomato, Arabidopsis and red kidney bean SlPAP homologs further validates the observation reported by Feder et al. (2019)[84]. For instance, presence of multiple histidine residues in vicinity of the active site and occurrence of atypical REKA domain, a characteristic of plant SlPAPs that possess phytase activity, and a conserved lysine residue (R337) in SlPAP15 homologs of these species is astonishing. Moreover, occurrence of residues such as asparagine and histidine residues (R283 and R284) and histidine (R374), considered to play an important role in substrate binding irrespective of the identity of the reactant, in the surrounding area of the reaction centre is also in accordance with the recent report [84]. Moreover, the presence of tyrosine residue (R230) close to di-nuclear centre suggests that it is likely to be involved in the interaction with Fe(III) and charge transfer [58]. Overall, the docking study provided very strong support to the results obtained by Feder et al.)2019) [84]. Nevertheless, it is intriguing that in spite of the presence of the characteristic amino acid residues, which are present in the already characterized SlPAP15 homologs with phytase activity in plants, and highly conserved asparagine and histidine residues (R283 and R284) and histidine (R374) residues, no such interaction between PEP or IHP and the protein could be established in our docking experiments. At least, this observation is partially supported by the fact that the purified AtPAP15 protein had shown weaker relative activity with Na-phytate during its biochemical characterization [245]. However, the lack of any interaction between SlPAP15 and PEP (a small molecule), even in multiple docking efforts, is still intriguing. To clear this ambiguity, we silenced *SlPAP15* using VIGS. At phenotypic level, although we didn't notice much difference in the silenced and unsilenced seedlings under both HP and LP conditions, but compromised biomass and lower soluble Pi and total P content was noticed in silenced seedlings. Surprisingly, we found lower PAE and phytase activity in silenced plants under both HP and LP conditions. Similar results were obtained in the *Slpap15*^{CR1} KO mutant lines. Apart from the loss of domain responsible for APase activity in the KO line, we also confirmed the loss of function of protein at biochemical level by performing In-gel assay and found abolished

SlPAP15 APase activity in the KO seedlings. The reduced total APase activity in *Slpap15*^{CR1} KO lines is in line with the reported reduced APase activity in shoot and root tissues of *atpap15* mutant seedlings [331]. We also noticed lower phytase activity in the KO lines under HP and LP conditions. Phytate is the main storage form of phosphate in seed and pollens, while to lesser extent it is also present in leaves [332]. High expression of SlPAP15 in fruit pulp and seeds is interesting. The primary composition of the globoid, found in the protein storage vacuole of the Arabidopsis seed embryo/pollen, consists of calcium/magnesium/potassium/phosphorus-phytate salts (phytin). The breakdown of phytin in these cells are crucial for the growth and division of the embryo [333][334]. These globoids exhibit changes in size and number during pollen maturation and germination [334]. Phytases are required during seed and pollen germination to hydrolyse phytate into less phosphorylated compounds, namely myoinositol and inorganic phosphate. This supply of phosphorus is essential for the development of seedlings [335]. Lower phytase activity of SlPAP15 in germinated seeds and leaves grown under normal condition confirms SlPAP15 having phytase activity. Thus, mutation in this gene might affect the seed germination due to poor conversion of phytate for releasing Pi. The decrease in phytase activity is in concurrent with the earlier published results in Arabidopsis, where pro-35S:AtPAP15 OX plants showed decreased total phytate content in leaves of transgenic Arabidopsis plants [73]. Depending on root phytase secretion, Phy-PAPs role in Pi acquisition varies among species. For instance, Arabidopsis roots do not release phytase, while tobacco roots release phytate PAPs [13,329,336,337]. This exemplifies Phy-PAPs dual role in PAE, breaking down external phytate and facilitating phosphate remobilization Wang et al. (2009) [329].In our study, PAE decreased in the *Slpap15*^{CR} KO lines. We further reconfirmed these results by supplying exogenous ATP as the sole P source, where lower uptake percentage of P in the KO lines supports *SlPAP15* role in Pi acquisition. This observation is supported in transgenic alfalfa, where improved P acquisition via phytase expressing gene was noticed [332]. To reconfirm the results obtained with *Slpap15*^{CR1} KO lines, we generated the 35S: SlPAP15-OX lines and noticed higher phytase activity in the leaves compared to the wild type. Altogether, we demonstrate that *SlPAP15* is a PSI gene with a dual function, exhibiting both acid phosphatase and phytase activity. We tried to express this protein in *E. coli* but failed and thus could not confirm the function and substrate of this gene directly with the recombinant protein. Interestingly, its regulation has been shown for the first time to be controlled by the SlSPX-SlPHR module. Given its impact on PAE, SlPAP15 holds potential for generating improved lines with enhanced Pi uptake potential.

Mycorrhizae, the symbiotic relationship between plant and fungus has been extensively studied for nutrients exchange and acquisition between the symbiotic partners. Among the key players involved in symbiosis, role of PAPs in increasing the availability of freely available phosphate in the rhizosphere is prominent. AM colonization mostly leads to uptake of nutrients from the rhizosphere by fungal partner and trading off them, such as of phosphate, in exchange of carbohydrate with the host. Most of the PSI genes such as PAPs are downregulated upon mycorrhization because of the sufficient uptake of Pi via fungal Pi transporters [338,339]. In soybean, it was found that more than 90 % of the PAPs are downregulated upon colonization [340]. Interestingly, higher expression of *GmPAP4* and *GmPAP33* in soybean, upon colonization has been reported [227]. Role of these genes in arbuscular degeneration has also been demonstrated via hydrolysis of phospholipids in mycorrhizal roots [341]. Hence, we are interested to find the role of tomato PAPs in this symbiotic relationship. Transcriptome of tomato roots colonized with Glomus species (*R. irregularis*) resulted in the identification of novel AM responsive PAPs in tomato [284]. Higher expression of these PAPs in the root tissue further indicates their AM-responsive nature. Among the highly expressed PAPs, we selected *SlPAP20* for its characterization. Transcripts of *SlPAP20* are more pronounced in roots and unopened flower buds. While its non-PSI nature further supports the argument that not all SlPAPs are Pi-starvation responsive. Silencing of *SlPAP20* resulted in the better seedlings growth phenotype upon AM colonization. However, it is still argumentative that how the silencing leads to better seedlings growth phenotype. Hence, we checked mycorrhization in the roots of the silenced seedlings. Interestingly, higher mycorrhization was noticed in the roots of the silenced seedlings than their EV controls. While there seems to be clear role of PAPs in influencing root mycorrhization by AM, further investigations are needed to unravel the mechanism/pathway responsible for the observed growth promotion in the silenced seedlings.

Summary

In conclusion, we for the first time reported the full complement of purple acid phosphatase genes in tomato and its wild relatives. Morpho-physiological and biochemical characterization studies show that cultivated tomato and *S. pimpinellifolium* seedlings differ from each other with regard to the degree of their PSR. It could be attributed to the inhibited induction of the root-associated PAPs transcripts in the wild relative. Homology modelling and docking studies reaffirms the importance of REKA domain and other key residues in facilitating SlPAP15 homologs interaction with its substrates, and also identifies other important residues, such as histidine (R365) in facilitating such interactions. Nevertheless, lack of any interaction of SlPAP15 with PEP, contrary to its Arabidopsis and red kidney bean homologs, and between tyrosine (R230) and Fe (III) of the di-nuclear centre site in the docking study warrants further investigations, especially the biochemical characterization of purified SlPAP15 protein with different substrates, to validate these observations. Altogether, the results presented here will help in rapid characterization of PAPs in Solanum clade and fast track the biotechnological efforts for improving phosphate use efficiency tomato, in future.

We screened 36 tomato accessions under low Pi availability and identified seven tolerant accessions based on their better performance under low phosphate availability. Seedlings of the tolerant varieties exhibited better photosynthetic efficiency and PUE, indicating better mobilization and recycling of Pi. We selected PR and AV (sensitive varieties), AVai, and CLN (tolerant varieties) for their detailed characterization. Avai and CLN accumulated lower ROS levels and exhibited lower SAP activity under LP conditions. Transcriptome analysis (PR vs CLN) identified different and commonly expressed sets of differentially expressed genes in the two varieties. Next, we identified 25 PAP members in the tomato genome. Expression profiling identified that the majority of them are PSI genes. The detailed characterization of three candidate PAPs (*SlPAP15, SlPAP17b* and *SlPAP26b*) identified their contribution to PSR in tomato plants. While *SlPAP26b* has a prominent role in Pi-homeostasis, *SlPAP15* seems to determine Pi uptake as *Slpap15*^{CR} KO lines were affected in plant SAP and PAE. A role of *SlPAP15* in controlling plant total phytase activity was also demonstrated. Interestingly, while *SlPAP15* is a PSI gene and is a direct target of PHR1/PHL1, the upregulation of *SlPAP26b* under Pi starvation seems to be independent of SlPHR1 and SlPHL1. Astonishingly, many of the non-PSI PAPs (*SlPAP9b*, *SlPAP18a*, and *SlPAP20*) were found to be induced in AM-colonized plants, expanding the roles of these enzymes beyond PSR. Silencing of *SlPAP20* further confirmed the vital role of this PAP in influencing plant growth by affecting the amplitude of mycorrhization in the colonized plants. In summary, the study identified Pi-tolerant tomato accessions and shed light on the newer functions of PAP enzymes during PSR and AM colonization in tomato plants. In our gene expression profiling experiment, *SlPAP15,* was consistently upregulated at multiple time points/tissues in the transcriptome data, indicating it to be a candidate PSI gene for further characterization. The 1-kb upstream promoter region of *SlPAP15* also harbored two P1BS elements, the binding site of PSR master regulator PHR1. Virus-Induced Gene silencing (VIGS) based characterization of this gene in tomato seedlings revealed lower root secretory acid phosphatase activity and lesser Pi accumulation, total P content, and Pi acquisition efficiency (PAE) in both Pi-sufficient and Pi-deficient seedlings. The silenced plants also accumulated higher levels of total anthocyanins and ROS than the empty vector (EV) control counterparts under Pi deficiency. Overall, *SlPAP15* silencing seemed to exacerbate Pi starvation response in these plants under LP conditions. The transient transactivation assay of this promoter in the presence of SlPHR1 and SlPHL1 in Nicotiana confirmed SlPAP15 to be a direct target of SlPHR1/SlPHL1 TFs, as indicated by the transactivation of pSlPAP15:GUS transcriptional fusion vector and overaccumulation of GUS protein in the leaf sectors co-bombarded with SlPHLs. We selected this gene for its further in-depth characterization using CRISPR/Cas9 gene-editing and overexpression approaches to reconfirm our VIGS results. The analysis of *Slpap15*^{CR1} KO plants revalidated the VIGS-based findings and confirmed its involvement in tomato seedlings PSR. However, *Slpap15*^{CR1} lines have lower PAE than WT, which we have confirmed by providing ATP as the only external P source. The mutation in the *SlPAP15* gene was also found to interfere with the seed germination ability of the *Slpap15*^{CR1} KO plants. In summary, we identified a PSI *SlPAP15* gene and provided evidence for its involvement in the alteration of PSR in tomato seedlings. Contrary to SlPAP15, we found that SlPAP26b is not a direct target of SlPHR1/SlPHL1 TFs in our transcriptional activation assay experiments. Unaltered *SlPAP26b* transcript levels in SlSPX1/2, and SlPHRR1/SlPHL1 silenced seedlings further supported this observation. VIGS-based silencing of *SlPAP26b* resulted in compromised growth of the silenced seedlings under both HP and LP conditions. Total soluble Pi and P content also reduced significantly in these seedlings under HP and LP conditions. We also noticed reduced SAP (secretory acid phosphatase activity) under LP conditions in *SlPAP26b* silenced seedlings, indicating this gene's involvement in controlling P homeostasis. We also characterized *SlPAP17b* for its contribution to PSR in tomato seedlings. Contrary to *SlPAP15* and *SlPAP26b*, silencing of *SlPAP17b* did not significantly change the seedlings phenotype under both conditions. Total soluble Pi and total P content in the seedlings also remained unaltered under both conditions. Interestingly, PUE was found to be considerably decreased in *SlPAP17b* silenced seedlings. The detailed characterization of three candidate SlPAP genes revealed the scale of their contribution to tomato seedlings' PSR. Our experiments also demonstrated that not all the SlPAPs are under the direct regulation of the SlSPX-SlPHR gene regulatory module and are directly controlled by SlPHR1/SlPHL1 TFs. The significant finding of the objective is that while *SlPAP15* has a major role in PAE, *SlPAP26b* is a major determinant of plant internal phosphate homeostasis in tomato plants. In conclusion, the study provides new insights into the distinct responses of plant genotypes under Pi starvation and sheds light on the role of candidate purple acid phosphatases in tomato seedlings PSR. Additionally, it demonstrates the association between these phosphatases and mycorrhization, highlighting the advantageous impact on plant growth and development.

Annexure 1: Presence of P1BS elements at 3-kb upstream region in all the SlPAPs.

S.No.	SlPAPs Gene	Gene IDs	PIBS number	PIBS location (bp)
1	SlPAP9b	Solyc01g068380.2.1	1	1187-1194bp at 5'
2	SlPAP10b	Solyc01g110060.2.1	1	2802-2809bp at 5'
3	SlPAP9a	Solyc04g005450.2.1	5	980-987bp / 1405-1412bp / 1572-1579bp /1755-1762bp /2839-2846bp at 5
4	SlPAP4	Solyc04g008260.2.1	2	2160-2167bp/2388-2395bp at 5'
5	SlPAP1	Solyc05g012260.2.1	1	1445-1452bp at 5'
6	SlPAP26b	Solyc07g007670.2.1	1	1356-1363bp at 5'
7	SlPAP27a	Solyc07g008550.2.1	0	Nil
8	SlPAP27b	Solyc07g008560.2.1	1	659-666bp at 5'
9	SlPAP27c	Solyc07g008570.2.1	1	1904-1911bp at 5'
10	SlPAP18a	Solyc07g064500.2.1	2	1372-1379bp/1876-1883bp at 5'
11	SlPAP24b	Solyc08g083250.2.1	1	1860-1867bp at 5'
12	SlPAP15	Solyc09g091910.1.1	3	1237-1244bp/2726-2733bp/2825-2832bp at 5'
13	SlPAP18b	Solyc10g006300.2.1	1	896-903bp at 5'
14	SlPAP26a	Solyc12g009800.1.1	1	2860-2867bp at 5'
15	SlPAP10a	Solyc01g110050.1.1	1	767-774bp at 5'
16	SlPAP17b	Solyc03g098010.2.1	2	2776-2783bp at 5' / 2811-2818bp at 5'
17	SlPAP7	Solyc04g008250.1.1	1	2889-2896bp at 5'
18	SlPAP24a	Solyc04g024640.1.1	1	1415-1422bp at 5'
19	SlPAP23a	Solyc04g051640.2.1	0	Nil
20	SlPAP23b	Solyc04g051650.2.1	1	717-724bp at 5'
21	SlPAP12	Solyc04g080920.1.1	2	816-823bp/2748-2755bp at 5'
22	SlPAP17a	Solyc06g072420.2.1	1	1421-1428bp at 5'
23	SlPAP25	Solyc07g053070.1.1	0	Nil
24	SlPAP21	Solyc09g009600.1.1	0	Nil
25	SlPAP20	Solyc09g009610.1.1	1	226-233bp at 5'

Annexure 2: Summary of the five conserved block with seven metal binding residues in tomato PAPs. Amino acid residues in bold are the metal binding.

S.No	SGN Loci	Gene name	1st BLOCK (**D**XG)	2nd BLOCK (G**D**XX**Y**)	3rd BLOCK (G**N**H(D/E)	4th BLOCK (VXX**H**)	5th BLOCK (G**H**X**H**)
1	Solyc05g012260	SlPAP1	DMG	GDIVY	GNHE	VTSH	GHVH
2	Solyc04g008260	SlPAP4	DWG	GDNFY	GNHD	VVGH	GHDH
3	Solyc04g008250	SlPAP7	DWG	GDNFY	GNHD	VIGH	absent
4	Solyc04g005450	SlPAP9a	DMG	GDISY	GNHE	VGNH	GHVH
5	Solyc01g068380	SlPAP9b	DFG	GDISY	GNHE	VTVH/VRVH	GHVH
6	Solyc01g110050	SlPAP10a	DLG	GDISY	GNHE	VLVH	GHVH
7	Solyc01g110060	SlPAP10b	DLG	GDLSY	GNHE	VLVH	GHVH
8	Solyc04g080920	SlPAP12	DTG	GDLSY	GNHE	VLMH	GHVH
9	Solyc09g091910	SlPAP15	DLG	GDLTY	GNHE	absent	GHVH
10	Solyc06g072420	SlPAP17a	DWG	GDNFY	GNHE	VLGH	GHDH
11	Solyc03g098010	SlPAP17b	DWG	GDNFY	GNHD	VVGH	GHDH
12	Solyc07g064500	SlPAP18a	DLG	GDLSY	GNHE	VLHH	GHVH
13	Solyc10g006300	SlPAP18b	DLG	GDLSY	GNHE	VLFH	GHVH
14	Solyc09g009610	SlPAP20	DLG	GDLSY	GNHE	VIVH	GHVH
15	Solyc09g009600	SlPAP21	DLG	GDLSY	GNHE	VIVH	GHVH
16	Solyc04g051640	SlPAP23a	DLG	GDQTY	absent	VTPH	absent
17	Solyc04g051650	SlPAP23b	DRG/DGG	GTQIY	GNHE	absent	GHVH
18	Solyc04g024640	SlPAP24a	DLG	absent	GNHE	VTEH	GHVH
19	Solyc08g083250	SlPAP24b	DMG	GDITY	GNHE	VTLH	GHVH
20	Solyc07g053070	SlPAP25	DLG	GDLSY	GNHE	VLLH	GHVH
21	Solyc12g009800	SlPAP26a	DLG	GDLSY	GNHE	VLMH	GHVH
22	Solyc07g007670	SlPAP26b	DLG	GDLSY	GNHD	VLMH	GHVH
23	Solyc07g008550	SlPAP27a	DMG	GDLPY	GNHE	absent	GHVH
24	Solyc07g008560	SlPAP27b	DMG	GDLPY	GNHE	absent	GHVH
25	Solyc07g008570	SlPAP27c	DMG	GDLPY	GNHE	absent	GHVH

Annexure 3: Predicted binding site in SlPAPs checked using RaptorX.

Gene name #	Pocket multiplicity	Binding residues	Top ligands	P-value	GDT
SlPAP9b	135	D335 D377 Y380 N410 H411 H499 I510 H538 H540	FE	3.32E-18	70
	61	T613 R614 S616 K634	NAG		
	50	L135 F278 W280	NAG		
	42	P214 S308 S309	NAG		
	39	F178 P340 R341 D342 P351 L354 K358	NAG		
	28	N213 P214	NAG		
SlPAP10b	159	D164 D193 Y196 N230 H231 H315 H325 H352 H354	FE	3.77E-15	82
	61	N424 N425 H428 W448	NAG		
	49	N110 N202	NAG		
	39	M40 S44 D45 V46 F169 N172 T176	NAG		
	39	Y53 H138	NAG		
SlPAP9a	137	D262 D305 Y308 N338 H339 H441 K452 H480 H482	FE	2.12E-17	370(63)
	61	T558 K559 E560 K561 E579	NAG		
	49	I206 G207 W208	NAG		
	42	K168 S236 R237	NAG		
	39	Y72 I90 T267 P268 E277 K280 K284	NAG		
	28	G141 R142	NAG		
SlPAP4	213	D46 D79 Y82 N117 H118 H211 H246 H248	Zn	3.50E-08	202(62)
	59	T299 T301 Q302 S319	NAG		
SlPAP1	138	D288 D329 Y332 N362 H363 H451 Y462 H493 H495	FE		
	65	F561 D562 N565 N583	NAG		
	49	C99 A101 W228	NAG		
	42	N258 T260 I262	NAG		
	39	L125 E293 A294 D295 P305 L308 K312	NAG		
SlPAP26b	153	D161 D188 Y191 N226 H227 H311 H321 H348 H350	FE	3.19E-14	357(75)
	61	K420 N421 H424 T444	NAG		
	50	K107 N198	NAG		
	39	I37 N41 E42 V43 L169 Q173	NAG		
	39	Y50 D135	NAG		
SlPAP27a	138	D308 D349 Y352 N382 H383 H471 Y482 H513 H515	FE	1.79E-22	499(80)
	49	C119 A121 W248	NAG		
	45	F581 N582 S585 T603	NAG		
	42	L276 N278 S280	NAG		
	39	F145 E313 R314 D315 P325 L328 D332	NAG		
	28	P180 K181 Y205	NAG		
SlPAP27b	138	D318 D359 Y362 N392 H393 H481 Y492 H523 H525	FE	500(87)	6.02E-23
	65	F591 D592 N595 T613	NAG		
	49	R245 S255 W258	NAG		
	42	L286 N288 V292	NAG		
	39	F155 E323 R324 D325 P335 L338 D342	NAG		
	28	P190 K191 Y215	NAG		
SlPAP27c	138	D312 D353 Y356 N386 H387 H475 W485 H517 H519	FE	4.89E-22	494(86)
	65	F585 D586 N589 T607	NAG		
	49	S249 V250 W252	NAG		
	42	N282 S284 V286	NAG		
	39	F155 E317 R318 D319 P329 L332 D336	NAG		
	28	P184 K185 Y209	NAG		
SlPAP18a	203	D155 D182 Y185 N215 H216 H298 H308 H335 H337	FE	5.92E-13	290(67)
	62	I396 N397 H400 S420 C429	NAG		
	43	F106	NAG		
	42	G160 K163 S164 D167 L199 P202	NAG		
SlPAP24b	138	D290 D331 Y334 N364 H365 H453 Y464 H495 H497	FE	3.31E-22	503(83)
	57	F563 N564 S567 T585	NAG		
	49	C101 A103 W230	NAG		
	42	L258 N260 S262	NAG		
	39	F127 E295 R296 D297 P307 L310 D314	NAG		
	28	P162 K163 Y187	NAG		
SlPAP15	207	D200 D227 Y230 N283 H284 H365 H375 H402 H404	FE	1.19E-13	296(60)
	62	K499 N500 H503 Y523	NAG		
	49	L140 Q141 N142	NAG		
SlPAP18b	217	D149 D176 Y179 N209 H210 H292 H302 H329 H331	FE	1.00E-12	285(65)
	66	V390 N391 H394 W414	NAG		
SlPAP26a	154	D161 D188 Y191 N226 H227 H311 H321 H348 H350	FE	2.01E-14	353(74)
	61	K420 N421 H424 V444	NAG		
	49	T107 Y197	NAG		
	41	I37 N41 E42 V43 Y166 L169 Q173	NAG		
	39	Y50 G134 D135	NAG		
SlPAP10a	159	D125 D154 Y157 N191 H192 H282 H292 H319 H321	FE	1.77E-15	375(87)
	65	K391 N392 H395 W415	NAG		
	48	Y70 N71 N163	NAG		
	42	M1 S5 D6 V7 N133 T137 R178	NAG		
	39	Y14 H99	NAG		
SlPAP17b	207	D41 D74 Y77 N112 H113 H205 H214 H240 H242	ZN	3.68E-09	204(63)
	58	T293 T295 H296 S313	NAG		
SlPAP7	198	D55 D88 Y91 N126 H127 H220 H229 I255 N257	FE	2.46E-06	150(57)
	41	Y62 S65 E66 I69 N109 A113	NAG		
SlPAP24a	220	H21 Y24 N76 H77 H133 W143 H175 H177	ZN	1.56E-07	172(77)
SlPAP23a	123	D194 D221 Y224 H277 M278 P279	FE	1.14E-08	142(51)
	50	L134 W135 N136	NAG		
	43	S61 A166 A167	NAG		
	39	L48 H52 P53 R54 S199 T202 D206	NAG		
	28	S61 E86 E99	NAG		
SlPAP23b	213	N16 H17 H98 S107 H108 H135 H137 I166	ZN	4.46E-09	195(72)
	61	V231 N232 H235 Y257	NAG		
SlPAP12	158	N168 D198 Y201 N235 H236 H320 H330 H357 H359	FE	2.55E-15	373(79)
	61	K429 N430 H433 W453	NAG		
	49	N114 F207	NAG		
	41	S48 D49 V50 H174 Q177 T181 R222	NAG		
	39	Y57 G141 N142	NAG		
SlPAP17a	219	D50 D83 Y86 N121 H122 H215 H224 H250 H252	ZN	3.51E-08	209(63)
SlPAP25	200	D157 D185 Y188 N222 H223 H307 H317 H344 H346	FE	1.02E-14	356(78)
	62	K416 N417 H420 W440	NAG		
	47	N101 F194	NAG		
	43	Y39 H130 N131	NAG		
SlPAP21	214	D150 D177 Y180 N210 H211 H294 H304 H331 H333	FE	2.01E-12	294(67)
	62	Y392 N393 H396 W417	NAG		
	43	F101	NAG		
SlPAP20	209	D149 D176 Y179 N209 H210 H293 H303 H332 H334	FE	7.89e-13	293(69)
	62	V393 N394 H397 W417	NAG		
	43	L99	NAG		

Annexure 4: Domain Organization in the *S. lycopersicum* PAP proteins.

S.No	Gene name	Gene IDs	Signal peptide (length, cleave site)	No. of N-glycosylation site	Subcellular Localization
1	SlPAP1	Solyc05g012260.2.1	16, VTS-HE	7	S
2	SlPAP4	Solyc04g008260.2.1	23, AMA-EL	1	S
3	SlPAP7	Solyc04g008250.1.1	Nil	2	S
4	SlPAP9a	Solyc04g005450.2.1	20, SSS-SQ	3	S
5	SlPAP9b	Solyc01g068380.2.1	25, CHS-FS	6	S
6	SlPAP10a	Solyc01g110050.1.1	Nil	5	S
7	SlPAP10b	Solyc01g110060.2.1	21, VLC-NG	5	S
8	SlPAP12	Solyc04g080920.1.1	19, VLS-TK	3	S
9	SlPAP15	Solyc09g091910.1.1	25, NEG-QI	7	S
10	SlPAP17a	Solyc06g072420.2.1	25, ASA-ST	2	S
11	SlPAP17b	Solyc03g098010.2.1	17, SRA-TG	1	S
12	SlPAP18a	Solyc07g064500.2.1	26, VRA-GE	Nil	S
13	SlPAP18b	Solyc10g006300.2.1	23, VLA-GD	2	S
14	SlPAP20	Solyc09g009610.1.1	23, SLS-YE	Nil	S
15	SlPAP21	Solyc09g009600.1.1	23, FNA-SP	2	S
16	SlPAP23a	Solyc04g051640.2.1	18, IFA-KR	4	S
17	SlPAP23b	Solyc04g051650.2.1	Nil	3	S
18	SlPAP24a	Solyc04g024640.1.1	Nil	2	S
19	SlPAP24b	Solyc08g083250.2.1	16, ASG-HS	6	S
20	SlPAP25	Solyc07g053070.1.1	18, CNG-GV	7	S
21	SlPAP26a	Solyc12g009800.1.1	20, GNA-GV	3	S
22	SlPAP26b	Solyc07g007670.2.1	20, GSA-GI	3	S
23	SlPAP27a	Solyc07g008550.2.1	19, VSC-EE	4	S
24	SlPAP27b	Solyc07g008560.2.1	25, VSA-QN	5	S
25	SlPAP27c	Solyc07g008570.2.1	25, ILA-QN	6	S

S.No	Gene name	SGN Loci	No of Transmembrane Helix	Length
1	SlPAP1	Solyc05g012260	Nil	606
2	SlPAP4	Solyc04g008260	1	328
3	SlPAP7	Solyc04g008250	1	264
4	SlPAP9a	Solyc04g005450	1	648
5	SlPAP9b	Solyc01g068380	1	639
6	SlPAP10a	Solyc01g110050	Nil	432
7	SlPAP10b	Solyc01g110060	Nil	465
8	SlPAP12	Solyc04g080920	Nil	471
9	SlPAP15	Solyc09g091910	1	555
10	SlPAP17a	Solyc06g072420	1	333
11	SlPAP17b	Solyc03g098010	Nil	323
12	SlPAP18a	Solyc07g064500	Nil	436
13	SlPAP18b	Solyc10g006300	1	438
14	SlPAP20	Solyc09g009610	1	425
15	SlPAP21	Solyc09g009600	Nil	441
16	SlPAP23a	Solyc04g051640	Nil	282
17	SlPAP23b	Solyc04g051650	Nil	271
18	SlPAP24a	Solyc04g024640	Nil	225
19	SlPAP24b	Solyc08g083250	Nil	608
20	SlPAP25	Solyc07g053070	Nil	456
21	SlPAP26a	Solyc12g009800	Nil	477
22	SlPAP26b	Solyc07g007670	Nil	477
23	SlPAP27a	Solyc07g008550	Nil	626
24	SlPAP27b	Solyc07g008560	1	636
25	SlPAP27c	Solyc07g008570	1	630

*** No GPI anchored sites are present in all the PAPs.**

Annexure 5: List of Primers used in the study

S.No.	Primer sequence		Gene ID	Gene name	Purpose
1	GGCTGCAATCAAGGAGGAA	FP		GAPDH	qPCR
	AAATCAATCACACGGGAACT	RP		GAPDH	qPCR
2	TCTCAACCCTAAGGCCAACAGAGAG	FP	Solyc11g005330	Actin	qPCR
	TCTCTCGGTGAGGATCTTCATCAGG	RP	Solyc11g005330	Actin	qPCR
3	TGGCGAACGATTTGCAAGTG	FP	Solyc09g090070	SlPT1	qPCR
	ATGCCAGCAATCACAATCGC	RP	Solyc09g090070	SlPT1	qPCR
4	TGCTGCTGTTTTCGCTATGC	FP	Solyc09g066410	SlPT7	qPCR
	TGCTTGAGGAACCGTTGAAC	RP	Solyc09g066410	SlPT7	qPCR
5	TTGCCGGCCTTGTTTCAATG	FP	Solyc06g051860	SlPT4	qPCR
	AGACGATCCGCCAGACATAATC	RP	Solyc06g051860	SlPT4	qPCR
6	GGAGAAACAAAGCCCTTCAAGG	FP	Solyc01g110050	SlPAP10a	qPCR
	TGCTGAAGCTCGCTTGATTG	RP	Solyc01g110050		qPCR
7	TGATTGGCGCAATGTGATGC	FP	Solyc03g098010	SlPAP17b	qPCR
	GTCCCACACTTCTAATGGCATG	RP	Solyc03g098010		qPCR
8	ATGGGAGAGATAGGGGAAAAGC	FP	Solyc04g008250	SlPAP7	qPCR
	CACGTCTTTTGCAAGCTAGGAG	RP	Solyc04g008250		qPCR
9	AAAGAACCTGGGAGAACAGTGG	FP	Solyc04g024640	SlPAP24a	qPCR
	TTGGCTGACGCAAAGCATTG	RP	Solyc04g024640		qPCR
10	AGCAGTTGTTGGTGACTTGG	FP	Solyc04g051640	SlPAP23a	qPCR
	TGAAGCTCCTTTACCACCAGTG	RP	Solyc04g051640		qPCR
11	AGTTGACGCTGATCATGCAG	FP	Solyc04g051650	SlPAP23b	qPCR
	TTTTCCTTTGGCAGGTCCAC	RP	Solyc04g051650		qPCR
12	ATGGAAGGGGAAACAATGCG	FP	Solyc04g080920	SlPAP12	qPCR
	TGACCAGCAAAGACAACGTC	RP	Solyc04g080920		qPCR
13	GCCATTAGAAGTGCAGGACATC	FP	Solyc06g072420	SlPAP17a	qPCR
	ATTGCAACGGGCTTTCAGTG	RP	Solyc06g072420		qPCR
14	TATGTTCCCGCGGAAAACAG	FP	Solyc07g008570	SlPAP27c	qPCR
	TTGGCTGACGCAAAGCATTG	RP	Solyc07g008570		qPCR
15	ATTGGGGATTTGGGGCAAAC	FP	Solyc07g053070	SlPAP25	qPCR
	AGCTTGGCCTTTTGGATTCG	RP	Solyc07g053070		qPCR
16	TGCTGACACACAACAACCAC	FP	Solyc09g009600	SlPAP21	qPCR
	TCGTGATTGCCATGTGTCAC	RP	Solyc09g009600		qPCR
17	TTTCAAAACCCCTCCAGCTG	FP	Solyc09g009610	SlPAP20	qPCR
	ACGTTGTCCAGTCAGTTTGC	RP	Solyc09g009610		qPCR
18	GCTGGTGGAACAAATGAGGT	FP	Solyc01g068380	SlPAP9b	qPCR
	CGAGAGGCAATTGGAGTGAT	RP	Solyc01g068380		qPCR
19	GGCAAGCAGTGTTGTTCGTA	FP	Solyc01g110060	SlPAP10b	qPCR
	TGTTTCCCCAATTTCAGGAG	RP	Solyc01g110060		qPCR
20	TCTGGATCGGGTTCAATTTC	FP	Solyc04g005450	SlPAP9a	qPCR
	TCCGACACAGCAAGAAGATG	RP	Solyc04g005450		qPCR
21	GGAGATTGGGGAAGAAGAGG	FP	Solyc04g008260	SlPAP4	qPCR
	TCATGGTTCCCCAAAACATT	RP	Solyc04g008260		qPCR
22	TGCTATTGCTGCTTTGGATG	FP	Solyc05g012260	SlPAP1	qPCR
	TTCAGGGTCACACGTAGCTG	RP	Solyc05g012260		qPCR
23	GTCCGTTGGGATACATTTGG	FP	Solyc07g007670	SlPAP26b	qPCR
	GCTCGATAAGGTGTGGGGTA	RP	Solyc07g007670		qPCR
24	CTGGTGGTTTGCAAAATCCT	FP	Solyc07g008550	SlPAP27a	qPCR
	GACGAATGGAACAGCCTCAT	RP	Solyc07g008550		qPCR
25	AGATTCGGGTGGAGAATGTG	FP	Solyc07g008560	SlPAP27b	qPCR
	CTATTGGCTGATGCAAAGCA	RP	Solyc07g008560		qPCR
26	CATGGGATCCACAATCTTCC	FP	Solyc07g064500	SlPAP18a	qPCR
	CTGTGCTGTCTTGCAATGGT	RP	Solyc07g064500		qPCR
27	GGTGGAGAATGTGGAGTGCT	FP	Solyc08g083250	SlPAP24b	qPCR
	TAACCAAGAACCCGATGAGC	RP	Solyc08g083250		qPCR
28	CTCATTTCCCGAAACTCCAA	FP	Solyc09g091910	SlPAP15	qPCR
	TATGCCTCCCGCATTAAAAG	RP	Solyc09g091910		qPCR
29	GGCCAGACTGGATGGACTAA	FP	Solyc10g006300	SlPAP18b	qPCR
	AAATGGCATCTTCCACCTTG	RP	Solyc10g006300		qPCR
30	TTACTCAGCTTTCCGCGAAT	FP	Solyc12g009800	SlPAP26a	qPCR
	ACGGAGACGATTTTGTTTCG	RP	Solyc12g009800		qPCR
31	AGCGGAAGTTCAACTGTCAG	FP	Solyc08g060920	SlSPX1	qPCR
	TGACCACTGGAGCGTTTTTC	RP	Solyc08g060920		qPCR
32	TTGCAGCAGCCTTTCTTCAC	FP	Solyc12g009480	SlSPX2	qPCR
	TTCATTTCCGTCAGCTTCCG	RP	Solyc12g009480		qPCR
33	ATAAGGCTGCGGAAATCAGG	FP	Solyc02g091590	SlSYMRK	qPCR
	ATAATGGATGGCGGCGTTTG	RP	Solyc02g091590		qPCR
34	AATCGGGCCACTGGAGATAA	FP	Solyc02g092820	SlGH3.4	qPCR
	TCTCGAAATTGCATAATCCATAAGTT	RP	Solyc02g092820		qPCR
35	TAAATGCCCCTGTCCAGAAC	FP	Solyc06g008200	SlPHR1	qPCR
	TTACAAGGTCAGCCCATTCC	RP	Solyc06g008200		qPCR
36	CAACAGCCCTTGGAGAAAAG	FP	Solyc09g072830	SlPHL1	qPCR
	CAGTTGCAGGATGTTGCTTG	RP	Solyc09g072830		qPCR

37	ATCATATGAAGTATTTTGGGGTTGTGG	FP	Solyc09g091910	SlPAP15	Overexpression
	CGGTCGACGATTAATTTTTTTATCAA	RP	Solyc09g091910	SlPAP15	Overexpression
38	TTTTGGTCTCAATTGCAGCATGTCCCCTAAAACTTGTTTTAGAGCTAGAAATAGC	FP	Solyc09g091910	SlPAP15	CRISPR
	TTTTGGTCTCAAAACCTTGGCATCCACCTTGGTACCAATCACTACTTCGACTC	RP	Solyc09g091910	SlPAP15	CRISPR
39	ATGAAGTATTTTGGGGTTGTGGGG	FP	Solyc09g091910	Exon-1	CRISPR in/del conf
	CCAAACAGAGTCATAAGTAGCAGAAAGG	RP	Solyc09g091910		
40	CTCAAATCATCGATCAGCTGGAATCTA	FP	Solyc09g091910	Exon-5	CRISPR in/del conf
	TGTGCTCTTGGATATCGGTACATTAGTC	RP	Solyc09g091910		
41	GTTACTCTAATTTTATTCGCCATTTAACCC	FP	Solyc09g091910	SlPAP15	PAP15 off target
	GGATATGTCTTTGGGTAGATACAGGTAAGGG	RP	Solyc09g091910	SlPAP15	PAP15 off target
42	ATCTAGATTGTAGCCAATTTGATATTCACCA	FP	Solyc09g091910	SlPAP15	VIGS
	AGGATCCTGAAGTATTTTGGGGTTGTGG	RP	Solyc09g091910	SlPAP15	VIGS
43	TAAAGCTTCACAACCTACGAAAAGTGAAAATGATCA	FP	Solyc09g091910	SlPAP15	Promoter
	GGATCCAATCAATCAAGAATTTGCATGCCCCA	RP	Solyc09g091910	SlPAP15	Promoter
44	ATGAATTCAGAATTTCACATCTTCACTGTTCAACCCT	FP	Solyc03g098010	SlPAP17b	VIGS
	ATGGATCCATATTCATGGTGGACACAACCCCA	RP	Solyc03g098010	SlPAP17b	VIGS
45	TATCTAGACATTTCCGATTTCATAGTAATACTTTGTGTCA	FP	Solyc12g009800	SlPAP26a	VIGS
	TAGGATCCATGTTGCTTCATCTCTTCTTTTCATTGTTC	RP	Solyc12g009800	SlPAP26a	VIGS
46	TATCTAGATTGCAGAATCTCCTTTTCCAATTTCATAG	FP	Solyc07g007670	SlPAP26b	VIGS
	TAGGATCCATGTTGCTTTGTCTCTTCTTTTCGTTATC	RP	Solyc07g007670	SlPAP26b	VIGS
47	GATCTAGA TGAAATTGAATTCTGGGCTTGTAAA	FP	Solyc09g009610	SlPAP20	VIGS
	TTGGATCC ATGGCTTCTAATAAGGTTAATATTTTGCTA	RP	Solyc09g009610	SlPAP20	VIGS

Annexure 6: Differentially expressed genes in RNA seq of Pusa Ruby (Sensitive variety).

S.No.	ID	Status	S.No.	ID	Status	S.No.	ID	Status
1	Solyc01g006540.4.1.10	Up regulated	51	Solyc01g102980.3.1.2	Up regulated	101	Solyc04g011600.4.1.2	Up regulated
2	Solyc07g049460.3.1.5	Up regulated	52	Solyc01g107540.4.1.2	Up regulated	102	Solyc04g008900.4.1.1	Up regulated
3	Solyc02g067750.4.1.1	Up regulated	53	Solyc01g108450.3.1.1	Up regulated	103	Solyc04g006970.4.1.1	Up regulated
4	Solyc01g109790.3.1.1	Up regulated	54	Solyc01g109610.4.1.2	Up regulated	104	Solyc04g007825.2.1.2	Up regulated
5	Solyc06g062560.2.1.4	Up regulated	55	Solyc03g044590.2.1.1	Up regulated	105	Solyc04g011940.3.1.1	Up regulated
6	Solyc09g091510.3.1.2	Up regulated	56	Solyc01g091760.3.1.1	Up regulated	106	Solyc04g055257.1.1.1	Up regulated
7	Solyc05g053550.3.1.2	Up regulated	57	Solyc01g094130.2.1.5	Up regulated	107	Solyc04g049340.3.1.6	Up regulated
8	Solyc10g075110.2.1.2	Up regulated	58	Solyc01g095960.4.1.2	Up regulated	108	Solyc04g064920.4.1.1	Up regulated
9	Solyc04g056620.2.1.5	Up regulated	59	Solyc01g105350.3.1.2	Up regulated	109	Solyc04g072050.4.1.9	Up regulated
10	Solyc11g065600.2.1.1	Up regulated	60	Solyc02g014730.3.1.2	Up regulated	110	Solyc04g079780.4.1.2	Up regulated
11	Solyc02g085020.4.1.6	Up regulated	61	Solyc02g031920.4.1.3	Up regulated	111	Solyc04g079890.4.1.2	Up regulated
12	Solyc07g049690.3.1.2	Up regulated	62	Solyc02g037550.3.1.2	Up regulated	112	Solyc05g007000.4.1.5	Up regulated
13	Solyc03g025190.4.1.2	Up regulated	63	Solyc02g032210.1.1.1	Up regulated	113	Solyc05g012350.4.1.10	Up regulated
14	Solyc06g061023.1.1.3	Up regulated	64	Solyc02g094400.4.1.4	Up regulated	114	Solyc05g012955.1.1.2	Up regulated
15	Solyc06g062550.4.1.4	Up regulated	65	Solyc02g092570.1.1.1	Up regulated	115	Solyc05g013010.3.1.2	Up regulated
16	Solyc06g069240.2.1.1	Up regulated	66	Solyc02g090340.2.1.2	Up regulated	116	Solyc05g014700.3.1.1	Up regulated
17	Solyc08g005670.2.1.2	Up regulated	67	Solyc02g090150.1.1.1	Up regulated	117	Solyc05g024260.3.1.6	Up regulated
18	Solyc08g005677.1.1.1	Up regulated	68	Solyc02g089170.4.1.4	Up regulated	118	Solyc05g026490.3.1.7	Up regulated
19	Solyc06g059930.4.1.2	Up regulated	69	Solyc02g086530.4.1.1	Up regulated	119	Solyc05g052370.3.1.1	Up regulated
20	Solyc06g071030.3.1.14	Up regulated	70	Solyc02g077300.2.1.3	Up regulated	120	Solyc05g046200.3.1.1	Up regulated
21	Solyc11g066580.3.1.3	Up regulated	71	Solyc02g071710.3.1.5	Up regulated	121	Solyc05g045670.4.1.1	Up regulated
22	Solyc06g062540.3.1.4	Up regulated	72	Solyc02g071050.4.1.1	Up regulated	122	Solyc05g051583.1.1.2	Up regulated
23	Solyc02g062400.3.1.2	Up regulated	73	Solyc02g069960.4.1.1	Up regulated	123	Solyc06g009240.4.1.1	Up regulated
24	Solyc02g085170.4.1.1	Up regulated	74	Solyc02g066800.3.1.1	Up regulated	124	Solyc06g009840.3.1.7	Up regulated
25	Solyc06g076920.3.1.1	Up regulated	75	Solyc02g065490.4.1.4	Up regulated	125	Solyc06g050900.3.1.4	Up regulated
26	Solyc09g082690.3.1.3	Up regulated	76	Solyc02g087540.3.1.1	Up regulated	126	Solyc06g051320.3.1.2	Up regulated
27	Solyc05g010320.4.1.4	Up regulated	77	Solyc02g085190.2.1.1	Up regulated	127	Solyc06g053220.3.1.1	Up regulated
28	Solyc07g054900.2.1.3	Up regulated	78	Solyc02g085150.4.1.3	Up regulated	128	Solyc06g053870.4.1.2	Up regulated
29	Solyc02g083860.3.1.2	Up regulated	79	Solyc02g083020.1.1.1	Up regulated	129	Solyc06g048860.3.1.5	Up regulated
30	Solyc07g055260.3.1.9	Up regulated	80	Solyc02g081770.2.1.1	Up regulated	130	Solyc06g076800.3.1.2	Up regulated
31	Solyc08g013820.3.1.2	Up regulated	81	Solyc02g079150.2.1.3	Up regulated	131	Solyc06g076760.2.1.5	Up regulated
32	Solyc05g054900.3.1.2	Up regulated	82	Solyc02g068610.2.1.1	Up regulated	132	Solyc06g076400.3.1.1	Up regulated
33	Solyc01g009330.1.1.1	Up regulated	83	Solyc03g005200.3.1.3	Up regulated	133	Solyc06g068130.3.1.1	Up regulated
34	Solyc01g007200.2.1.1	Up regulated	84	Solyc03g007710.3.1.2	Up regulated	134	Solyc06g066840.3.1.3	Up regulated
35	Solyc01g058160.3.1.1	Up regulated	85	Solyc03g019690.1.1.1	Up regulated	135	Solyc06g064620.3.1.5	Up regulated
36	Solyc01g058030.2.1.1	Up regulated	86	Solyc03g025430.1.1.1	Up regulated	136	Solyc06g062370.4.1.3	Up regulated
37	Solyc01g067295.1.1.1	Up regulated	87	Solyc03g078500.3.1.2	Up regulated	137	Solyc06g059710.3.1.2	Up regulated
38	Solyc01g065740.3.1.4	Up regulated	88	Solyc03g093180.1.1.1	Up regulated	138	Solyc06g074680.4.1.6	Up regulated
39	Solyc01g081600.3.1.2	Up regulated	89	Solyc03g098010.3.1.7	Up regulated	139	Solyc06g074640.2.1.9	Up regulated
40	Solyc01g088430.4.1.10	Up regulated	90	Solyc03g098700.1.1.1	Up regulated	140	Solyc06g063280.1.1.1	Up regulated
41	Solyc01g090210.4.1.4	Up regulated	91	Solyc03g098780.2.1.1	Up regulated	141	Solyc06g082240.2.1.5	Up regulated
42	Solyc01g090790.4.1.5	Up regulated	92	Solyc03g097760.2.1.1	Up regulated	142	Solyc07g007620.3.1.1	Up regulated
43	Solyc01g090890.3.1.3	Up regulated	93	Solyc03g098710.1.1.1	Up regulated	143	Solyc07g008210.4.1.3	Up regulated
44	Solyc01g094070.3.1.2	Up regulated	94	Solyc03g122140.3.1.1	Up regulated	144	Solyc07g008710.3.1.2	Up regulated
45	Solyc01g094870.3.1.8	Up regulated	95	Solyc03g121680.3.1.3	Up regulated	145	Solyc07g009340.2.1.3	Up regulated
46	Solyc01g094360.3.1.3	Up regulated	96	Solyc03g121420.4.1.4	Up regulated	146	Solyc07g016080.4.1.1	Up regulated
47	Solyc01g097470.4.1.1	Up regulated	97	Solyc07g014600.2.1.2	Up regulated	147	Solyc07g054790.1.1.1	Up regulated
48	Solyc01g098570.2.1.1	Up regulated	98	Solyc03g119590.1.1.1	Up regulated	148	Solyc08g068480.1.1.1	Up regulated
49	Solyc01g102890.4.1.1	Up regulated	99	Solyc03g111815.1.1.1	Up regulated	149	Solyc07g054580.3.1.1	Up regulated
50	Solyc01g102910.4.1.2	Up regulated	100	Solyc04g015120.3.1.1	Up regulated	150	Solyc07g045140.4.1.10	Up regulated

S.No.	ID	Status	S.No.	ID	Status	S.No.	ID	Status
151	Solyc07g042400.2.1.2	Up regulated	201	Solyc10g085870.1.1.1	Up regulated	251	Solyc12g098130.1.1.1	Up regulated
152	Solyc07g064760.2.1.2	Up regulated	202	Solyc10g085650.2.1.2	Up regulated	252	Solyc02g092070.3.1.4	Up regulated
153	Solyc07g062510.2.1.5	Up regulated	203	Solyc11g007250.2.1.1	Up regulated	253	Solyc02g084034.1.1.3	Up regulated
154	Solyc07g061800.4.1.1	Up regulated	204	Solyc11g008260.2.1.1	Up regulated	254	Solyc02g070820.1.1.1	Up regulated
155	Solyc07g063500.4.1.1	Up regulated	205	Solyc11g017100.1.1.1	Up regulated	255	Solyc02g065530.4.1.2	Up regulated
156	Solyc08g006020.4.1.3	Up regulated	206	Solyc11g044450.3.1.1	Up regulated	256	Solyc02g065020.2.1.1	Up regulated
157	Solyc08g007210.4.1.1	Up regulated	207	Solyc11g066640.1.1.1	Up regulated	257	Solyc03g046580.1.1.1	Up regulated
158	Solyc03g078320.1.1.1	Up regulated	208	Solyc11g071290.2.1.4	Up regulated	258	Solyc03g123940.1.1.2	Up regulated
159	Solyc08g066260.3.1.6	Up regulated	209	Solyc11g071940.1.1.1	Up regulated	259	Solyc03g119620.1.1.3	Up regulated
160	Solyc08g066220.4.1.6	Up regulated	210	Solyc11g072060.3.1.4	Up regulated	260	Solyc03g116250.2.1.1	Up regulated
161	Solyc08g068720.1.1.1	Up regulated	211	Solyc12g005020.2.1.5	Up regulated	261	Solyc03g111060.3.1.1	Up regulated
162	Solyc08g081820.4.1.3	Up regulated	212	Solyc12g006805.1.1.1	Up regulated	262	Solyc04g016490.4.1.1	Up regulated
163	Solyc08g079010.1.1.1	Up regulated	213	Solyc12g008520.2.1.3	Up regulated	263	Solyc04g016250.3.1.3	Up regulated
164	Solyc08g078890.3.1.1	Up regulated	214	Solyc12g008830.3.1.1	Up regulated	264	Solyc04g005600.3.1.3	Up regulated
165	Solyc08g077910.3.1.3	Up regulated	215	Solyc12g009480.2.1.1	Up regulated	265	Solyc04g005410.3.1.4	Up regulated
166	Solyc08g077170.3.1.5	Up regulated	216	Solyc12g011010.2.1.2	Up regulated	266	Solyc04g063370.3.1.5	Up regulated
167	Solyc08g076710.3.1.1	Up regulated	217	Solyc12g036440.1.1.1	Up regulated	267	Solyc04g081755.2.1.4	Up regulated
168	Solyc08g076700.1.1.1	Up regulated	218	Solyc12g036480.2.1.1	Up regulated	268	Solyc05g005865.1.1.1	Up regulated
169	Solyc12g039080.3.1.3	Up regulated	219	Solyc12g044840.2.1.4	Up regulated	269	Solyc05g052240.3.1.4	Up regulated
170	Solyc09g005940.4.1.8	Up regulated	220	Solyc12g041960.2.1.2	Up regulated	270	Solyc05g051450.2.1.4	Up regulated
171	Solyc09g005970.1.1.1	Up regulated	221	Solyc12g044390.3.1.1	Up regulated	271	Solyc06g060320.4.1.2	Up regulated
172	Solyc09g010810.3.1.6	Up regulated	222	Solyc12g056760.2.1.2	Up regulated	272	Solyc07g006620.4.1.6	Up regulated
173	Solyc09g007570.3.1.2	Up regulated	223	Solyc12g056710.3.1.1	Up regulated	273	Solyc07g008570.3.1.11	Up regulated
174	Solyc09g065420.3.1.3	Up regulated	224	Solyc12g056610.3.1.3	Up regulated	274	Solyc07g061760.3.1.2	Up regulated
175	Solyc09g066410.3.1.1	Up regulated	225	Solyc12g088510.1.1.1	Up regulated	275	Solyc08g014190.4.1.1	Up regulated
176	Solyc09g064490.3.1.1	Up regulated	226	Solyc12g100280.3.1.2	Up regulated	276	Solyc08g066620.3.1.3	Up regulated
177	Solyc09g075990.3.1.1	Up regulated	227	Solyc12g100180.2.1.19	Up regulated	277	Solyc08g078030.4.1.1	Up regulated
178	Solyc09g075700.1.1.1	Up regulated	228	Solyc06g073587.1.1.9	Up regulated	278	Solyc09g011980.1.1.1	Up regulated
179	Solyc09g082280.3.1.3	Up regulated	229	Solyc03g112090.3.1.1	Up regulated	279	Solyc09g011080.3.1.2	Up regulated
180	Solyc09g083330.3.1.1	Up regulated	230	Solyc07g064750.1.1.2	Up regulated	280	Solyc09g007470.2.1.1	Up regulated
181	Solyc09g084490.4.1.3	Up regulated	231	Solyc01g109010.3.1.1	Up regulated	281	Solyc09g065100.3.1.6	Up regulated
182	Solyc09g089650.1.1.1	Up regulated	232	Solyc09g065350.1.1.1	Up regulated	282	Solyc01g095510.3.1.10	Up regulated
183	Solyc09g089730.3.1.1	Up regulated	233	Solyc04g015630.2.1.1	Up regulated	283	Solyc09g098010.3.1.3	Up regulated
184	Solyc09g090005.1.1.1	Up regulated	234	Solyc03g150149.1.1.1	Up regulated	284	Solyc10g008930.1.1.1	Up regulated
185	Solyc09g091880.4.1.6	Up regulated	235	Solyc12g006130.3.1.1	Up regulated	285	Solyc10g047040.3.1.2	Up regulated
186	Solyc09g092560.4.1.3	Up regulated	236	Solyc09g092750.3.1.2	Up regulated	286	Solyc10g009540.1.1.4	Up regulated
187	Solyc09g092620.3.1.2	Up regulated	237	Solyc02g022900.3.1.2	Up regulated	287	Solyc12g049616.1.1.1	Up regulated
188	Solyc09g098490.4.1.2	Up regulated	238	Solyc02g064810.1.1.3	Up regulated	288	Solyc02g063000.4.1.1	Up regulated
189	Solyc10g007940.1.1.1	Up regulated	239	Solyc04g078350.1.1.1	Up regulated	289	Solyc03g013250.4.1.6	Up regulated
190	Solyc10g005360.4.1.3	Up regulated	240	Solyc11g069730.1.1.1	Up regulated	290	Solyc04g008245.1.1.7	Up regulated
191	Solyc10g009590.4.1.1	Up regulated	241	Solyc10g012000.1.1.1	Up regulated	291	Solyc08g068490.3.1.1	Up regulated
192	Solyc10g055780.1.1.1	Up regulated	242	Solyc04g057790.1.1.3	Up regulated	292	Solyc07g016210.1.1.2	Up regulated
193	Solyc10g055250.1.1.1	Up regulated	243	Solyc10g008780.1.1.1	Up regulated	293	Solyc08g060920.4.1.3	Up regulated
194	Solyc10g054910.1.1.1	Up regulated	244	Solyc01g100070.3.1.1	Up regulated	294	Solyc08g065410.3.1.1	Up regulated
195	Solyc10g048030.2.1.1	Up regulated	245	Solyc01g098310.3.1.2	Up regulated	295	Solyc01g088260.3.1.1	Up regulated
196	Solyc10g079350.3.1.1	Up regulated	246	Solyc11g005970.1.1.1	Up regulated	296	Solyc08g067940.3.1.1	Up regulated
197	Solyc10g080870.3.1.2	Up regulated	247	Solyc11g018710.1.1.1	Up regulated	297	Solyc10g055790.1.1.1	Up regulated
198	Solyc10g083440.2.1.1	Up regulated	248	Solyc11g066790.2.1.20	Up regulated	298	Solyc06g005460.1.1.1	Up regulated
199	Solyc10g084890.3.1.1	Up regulated	249	Solyc11g071670.2.1.1	Up regulated	299	Solyc09g057760.4.1.2	Up regulated
200	Solyc10g085100.2.1.1	Up regulated	250	Solyc12g099860.2.1.9	Up regulated	300	Solyc01g099250.3.1.4	Up regulated

S.No.	ID	Status	S.No.	ID	Status	S.No.	ID	Status
301	Solyc03g053085.1.1.3	Up regulated	316	Solyc01g106953.1.1.1	Up regulated	331	Solyc09g097800.2.1.3	Up regulated
302	Solyc01g081160.4.1.6	Up regulated	317	Solyc12g042110.3.1.1	Up regulated	332	Solyc10g075090.3.1.2	Up regulated
303	Solyc11g006505.1.1.2	Up regulated	318	Solyc01g009890.1.1.1	Up regulated	333	Solyc10g075103.1.1.1	Up regulated
304	Solyc12g036810.3.1.16	Up regulated	319	Solyc02g070600.3.1.1	Up regulated	334	Solyc11g044453.1.1.1	Up regulated
305	Solyc02g062045.1.1.1	Up regulated	320	Solyc02g089780.3.1.1	Up regulated	335	Solyc07g005630.3.1.1	Up regulated
306	Solyc02g065480.2.1.1	Up regulated	321	Solyc03g083170.3.1.11	Up regulated	336	Solyc00g014790.2.1.1	Up regulated
307	Solyc02g079550.3.1.1	Up regulated	322	Solyc03g111885.1.1.1	Up regulated			
308	Solyc03g077980.2.1.5	Up regulated	323	Solyc03g114200.4.1.6	Up regulated			
309	Solyc03g098720.3.1.2	Up regulated	324	Solyc03g118770.4.1.1	Up regulated			
310	Solyc04g008910.3.1.5	Up regulated	325	Solyc04g014330.1.1.1	Up regulated			
311	Solyc04g015110.3.1.2	Up regulated	326	Solyc05g006730.4.1.2	Up regulated			
312	Solyc05g055750.3.1.1	Up regulated	327	Solyc05g012950.3.1.1	Up regulated			
313	Solyc07g051940.4.1.1	Up regulated	328	Solyc05g051345.1.1.2	Up regulated			
314	Solyc08g080040.4.1.3	Up regulated	329	Solyc11g021360.3.1.1	Up regulated			
315	Solyc10g049870.1.1.1	Up regulated	330	Solyc01g097930.3.1.5	Up regulated			

S.No.	ID	Status	S.No.	ID	Status	S.No.	ID	Status
1	Solyc03g093390.4.1.1	Downregulated	61	Solyc01g098720.3.1.2	Downregulated	121	Solyc04g008120.3.1.1	Downregulated
2	Solyc01g105880.4.1.7	Downregulated	62	Solyc01g099050.3.1.2	Downregulated	122	Solyc04g007760.3.1.2	Downregulated
3	Solyc03g044790.3.1.3	Downregulated	63	Solyc01g099210.3.1.9	Downregulated	123	Solyc04g005170.1.1.1	Downregulated
4	Solyc08g078900.1.1.1	Downregulated	64	Solyc01g106510.2.1.1	Downregulated	124	Solyc04g005140.2.1.1	Downregulated
5	Solyc08g068610.3.1.4	Downregulated	65	Solyc01g108860.3.1.3	Downregulated	125	Solyc04g011980.1.1.1	Downregulated
6	Solyc12g096750.2.1.1	Downregulated	66	Solyc01g110780.1.1.1	Downregulated	126	Solyc04g008670.2.1.1	Downregulated
7	Solyc10g055260.2.1.1	Downregulated	67	Solyc01g109390.3.1.1	Downregulated	127	Solyc04g051490.3.1.13	Downregulated
8	Solyc03g120060.2.1.5	Downregulated	68	Solyc01g090300.2.1.1	Downregulated	128	Solyc04g071520.3.1.7	Downregulated
9	Solyc02g081120.4.1.1	Downregulated	69	Solyc01g091100.2.1.1	Downregulated	129	Solyc04g072920.4.1.1	Downregulated
10	Solyc01g105070.3.1.1	Downregulated	70	Solyc01g109320.4.1.6	Downregulated	130	Solyc04g074810.4.1.3	Downregulated
11	Solyc02g091990.3.1.1	Downregulated	71	Solyc01g111010.4.1.3	Downregulated	131	Solyc04g074880.2.1.1	Downregulated
12	Solyc06g005465.1.1.3	Downregulated	72	Solyc02g092410.3.1.7	Downregulated	132	Solyc04g077990.3.1.2	Downregulated
13	Solyc04g058100.3.1.3	Downregulated	73	Solyc02g090220.3.1.1	Downregulated	133	Solyc04g079130.2.1.3	Downregulated
14	Solyc01g095080.3.1.1	Downregulated	74	Solyc02g089300.3.1.1	Downregulated	134	Solyc04g079260.3.1.3	Downregulated
15	Solyc02g087970.1.1.1	Downregulated	75	Solyc02g088270.3.1.5	Downregulated	135	Solyc04g078170.2.1.4	Downregulated
16	Solyc02g085500.4.1.1	Downregulated	76	Solyc02g088210.3.1.3	Downregulated	136	Solyc05g008670.3.1.2	Downregulated
17	Solyc01g005160.4.1.1	Downregulated	77	Solyc02g087170.4.1.1	Downregulated	137	Solyc05g009700.4.1.5	Downregulated
18	Solyc04g050440.3.1.1	Downregulated	78	Solyc02g086452.1.1.2	Downregulated	138	Solyc05g010060.4.1.3	Downregulated
19	Solyc10g076610.2.1.7	Downregulated	79	Solyc02g086300.4.1.3	Downregulated	139	Solyc05g010800.3.1.1	Downregulated
20	Solyc07g056670.3.1.3	Downregulated	80	Solyc02g085630.3.1.2	Downregulated	140	Solyc05g012030.1.1.1	Downregulated
21	Solyc08g080670.1.1.1	Downregulated	81	Solyc02g083490.3.1.4	Downregulated	141	Solyc05g012320.1.1.2	Downregulated
22	Solyc01g104740.3.1.1	Downregulated	82	Solyc02g083480.3.1.4	Downregulated	142	Solyc05g015880.3.1.4	Downregulated
23	Solyc07g008240.3.1.1	Downregulated	83	Solyc02g082170.3.1.1	Downregulated	143	Solyc05g007230.4.1.2	Downregulated
24	Solyc12g008650.2.1.6	Downregulated	84	Solyc02g080200.4.1.1	Downregulated	144	Solyc05g007610.2.1.1	Downregulated
25	Solyc11g011210.2.1.1	Downregulated	85	Solyc02g079460.1.1.1	Downregulated	145	Solyc05g007630.3.1.3	Downregulated
26	Solyc01g067370.3.1.1	Downregulated	86	Solyc02g077520.3.1.1	Downregulated	146	Solyc05g008320.1.1.1	Downregulated
27	Solyc07g061720.3.1.3	Downregulated	87	Solyc02g077400.4.1.1	Downregulated	147	Solyc05g055290.3.1.4	Downregulated
28	Solyc01g087850.2.1.1	Downregulated	88	Solyc02g076910.3.1.3	Downregulated	148	Solyc05g054780.3.1.1	Downregulated
29	Solyc08g014000.3.1.1	Downregulated	89	Solyc02g076820.4.1.1	Downregulated	149	Solyc05g054090.3.1.2	Downregulated
30	Solyc01g090810.4.1.4	Downregulated	90	Solyc02g071070.3.1.4	Downregulated	150	Solyc05g053860.4.1.1	Downregulated
31	Solyc09g064910.1.1.1	Downregulated	91	Solyc02g070890.3.1.1	Downregulated	151	Solyc05g053020.3.1.2	Downregulated
32	Solyc10g007960.1.1.1	Downregulated	92	Solyc02g070180.3.1.1	Downregulated	152	Solyc05g052890.3.1.5	Downregulated
33	Solyc03g120380.3.1.1	Downregulated	93	Solyc02g066960.3.1.2	Downregulated	153	Solyc05g052870.4.1.2	Downregulated
34	Solyc11g044910.2.1.1	Downregulated	94	Solyc02g065600.3.1.1	Downregulated	154	Solyc05g052670.1.1.1	Downregulated
35	Solyc06g074990.3.1.3	Downregulated	95	Solyc02g064970.4.1.4	Downregulated	155	Solyc05g052650.2.1.1	Downregulated
36	Solyc06g073590.3.1.2	Downregulated	96	Solyc02g064830.4.1.1	Downregulated	156	Solyc05g051220.3.1.13	Downregulated
37	Solyc10g084120.2.1.4	Downregulated	97	Solyc02g090450.3.1.4	Downregulated	157	Solyc05g046010.4.1.1	Downregulated
38	Solyc07g014680.4.1.1	Downregulated	98	Solyc02g068540.2.1.1	Downregulated	158	Solyc05g055030.2.1.1	Downregulated
39	Solyc12g011030.3.1.3	Downregulated	99	Solyc02g063480.2.1.2	Downregulated	159	Solyc06g008930.3.1.2	Downregulated
40	Solyc01g101210.4.1.6	Downregulated	100	Solyc03g006290.4.1.1	Downregulated	160	Solyc06g034370.1.1.1	Downregulated
41	Solyc08g079920.2.1.1	Downregulated	101	Solyc03g006910.3.1.2	Downregulated	161	Solyc06g053930.3.1.4	Downregulated
42	Solyc04g014510.3.1.12	Downregulated	102	Solyc03g124110.2.1.1	Downregulated	162	Solyc06g076330.3.1.2	Downregulated
43	Solyc08g080660.1.1.1	Downregulated	103	Solyc03g031530.4.1.1	Downregulated	163	Solyc06g075970.4.1.1	Downregulated
44	Solyc04g011880.1.1.1	Downregulated	104	Solyc03g031590.4.1.2	Downregulated	164	Solyc06g075480.4.1.1	Downregulated
45	Solyc08g074682.1.1.1	Downregulated	105	Solyc03g034320.3.1.3	Downregulated	165	Solyc06g073580.4.1.3	Downregulated
46	Solyc03g044180.1.1.1	Downregulated	106	Solyc03g046380.1.1.1	Downregulated	166	Solyc06g070910.3.1.1	Downregulated
47	Solyc04g078710.3.1.2	Downregulated	107	Solyc03g080100.4.1.5	Downregulated	167	Solyc06g065010.4.1.1	Downregulated
48	Solyc01g090310.3.1.1	Downregulated	108	Solyc03g079980.2.1.2	Downregulated	168	Solyc06g060500.1.1.1	Downregulated
49	Solyc01g009760.3.1.1	Downregulated	109	Solyc03g078620.1.1.1	Downregulated	169	Solyc06g069900.3.1.1	Downregulated
50	Solyc01g006310.3.1.4	Downregulated	110	Solyc03g082470.4.1.1	Downregulated	170	Solyc06g063430.2.1.1	Downregulated
51	Solyc01g006580.4.1.8	Downregulated	111	Solyc03g093110.3.1.3	Downregulated	171	Solyc06g063200.2.1.2	Downregulated
52	Solyc01g005390.3.1.5	Downregulated	112	Solyc03g121100.4.1.3	Downregulated	172	Solyc06g084190.3.1.1	Downregulated
53	Solyc01g006055.1.1.1	Downregulated	113	Solyc03g119390.4.1.1	Downregulated	173	Solyc06g084140.4.1.1	Downregulated
54	Solyc01g005320.2.1.1	Downregulated	114	Solyc03g115040.4.1.2	Downregulated	174	Solyc06g083160.1.1.1	Downregulated
55	Solyc01g009320.2.1.1	Downregulated	115	Solyc03g114900.3.1.3	Downregulated	175	Solyc07g005380.4.1.2	Downregulated
56	Solyc01g057320.3.1.17	Downregulated	116	Solyc03g111300.1.1.1	Downregulated	176	Solyc07g008230.1.1.1	Downregulated
57	Solyc01g066910.3.1.1	Downregulated	117	Solyc04g015610.3.1.3	Downregulated	177	Solyc07g017880.4.1.1	Downregulated
58	Solyc01g073860.3.1.1	Downregulated	118	Solyc04g011480.3.1.1	Downregulated	178	Solyc07g005150.3.1.1	Downregulated
59	Solyc01g081250.3.1.3	Downregulated	119	Solyc04g010330.3.1.3	Downregulated	179	Solyc07g005170.3.1.1	Downregulated
60	Solyc01g088360.4.1.1	Downregulated	120	Solyc04g008970.3.1.4	Downregulated	180	Solyc07g006740.3.1.6	Downregulated

S.No.	ID	Status	S.No.	ID	Status	S.No.	ID	Status
181	Solyc07g006800.1.1.1	Downregulated	241	Solyc09g090300.3.1.2	Downregulated	301	Solyc09g008500.3.1.1	Downregulated
182	Solyc07g006850.3.1.5	Downregulated	242	Solyc09g090900.4.1.15	Downregulated	302	Solyc02g085770.4.1.4	Downregulated
183	Solyc07g055990.3.1.3	Downregulated	243	Solyc09g092600.3.1.1	Downregulated	303	Solyc04g009590.4.1.2	Downregulated
184	Solyc07g053840.1.1.1	Downregulated	244	Solyc09g089910.1.1.1	Downregulated	304	Solyc11g069900.3.1.1	Downregulated
185	Solyc07g048000.3.1.3	Downregulated	245	Solyc09g091950.1.1.2	Downregulated	305	Solyc08g082380.1.1.1	Downregulated
186	Solyc07g042520.4.1.3	Downregulated	246	Solyc10g008120.4.1.1	Downregulated	306	Solyc02g089460.1.1.1	Downregulated
187	Solyc07g042510.3.1.4	Downregulated	247	Solyc10g007880.4.1.5	Downregulated	307	Solyc02g092890.1.1.1	Downregulated
188	Solyc07g041500.3.1.5	Downregulated	248	Solyc10g005010.4.1.1	Downregulated	308	Solyc02g068460.1.1.1	Downregulated
189	Solyc07g052620.1.1.1	Downregulated	249	Solyc10g009310.4.1.2	Downregulated	309	Solyc11g045140.1.1.4	Downregulated
190	Solyc07g051890.1.1.1	Downregulated	250	Solyc10g005140.1.1.1	Downregulated	310	Solyc11g069880.1.1.1	Downregulated
191	Solyc07g062480.2.1.1	Downregulated	251	Solyc10g017510.3.1.2	Downregulated	311	Solyc01g100440.3.1.1	Downregulated
192	Solyc08g006520.1.1.1	Downregulated	252	Solyc10g052600.1.1.1	Downregulated	312	Solyc01g102550.4.1.9	Downregulated
193	Solyc08g007430.2.1.5	Downregulated	253	Solyc10g076240.3.1.1	Downregulated	313	Solyc01g110730.4.1.1	Downregulated
194	Solyc08g007480.2.1.2	Downregulated	254	Solyc10g076830.2.1.2	Downregulated	314	Solyc02g094370.1.1.1	Downregulated
195	Solyc08g008360.2.1.1	Downregulated	255	Solyc10g078230.3.1.1	Downregulated	315	Solyc02g093960.3.1.11	Downregulated
196	Solyc08g008600.3.1.2	Downregulated	256	Solyc10g078720.2.1.7	Downregulated	316	Solyc02g092460.3.1.5	Downregulated
197	Solyc08g008660.4.1.1	Downregulated	257	Solyc10g083250.2.1.3	Downregulated	317	Solyc02g090410.4.1.1	Downregulated
198	Solyc08g007600.2.1.1	Downregulated	258	Solyc10g083880.2.1.2	Downregulated	318	Solyc02g089790.4.1.16	Downregulated
199	Solyc08g007610.1.1.1	Downregulated	259	Solyc10g083890.2.1.6	Downregulated	319	Solyc02g086760.1.1.1	Downregulated
200	Solyc08g007660.2.1.2	Downregulated	260	Solyc10g085090.3.1.5	Downregulated	320	Solyc02g072220.1.1.1	Downregulated
201	Solyc08g014050.2.1.1	Downregulated	261	Solyc10g080060.1.1.1	Downregulated	321	Solyc03g034410.1.1.1	Downregulated
202	Solyc08g014350.1.1.1	Downregulated	262	Solyc10g086490.2.1.4	Downregulated	322	Solyc03g078090.4.1.4	Downregulated
203	Solyc08g036620.4.1.3	Downregulated	263	Solyc11g007370.3.1.1	Downregulated	323	Solyc03g121230.3.1.14	Downregulated
204	Solyc08g041700.1.1.1	Downregulated	264	Solyc11g010290.2.1.4	Downregulated	324	Solyc03g112950.4.1.21	Downregulated
205	Solyc08g044510.4.1.7	Downregulated	265	Solyc11g011630.3.1.1	Downregulated	325	Solyc04g008470.3.1.2	Downregulated
206	Solyc08g067510.1.1.1	Downregulated	266	Solyc11g011730.1.1.1	Downregulated	326	Solyc04g008080.1.1.1	Downregulated
207	Solyc08g068600.3.1.4	Downregulated	267	Solyc11g018500.3.1.1	Downregulated	327	Solyc04g054840.1.1.1	Downregulated
208	Solyc08g068630.4.1.1	Downregulated	268	Solyc11g005300.2.1.1	Downregulated	328	Solyc04g054440.3.1.2	Downregulated
209	Solyc08g061010.4.1.1	Downregulated	269	Solyc11g005860.2.1.5	Downregulated	329	Solyc04g074930.4.1.1	Downregulated
210	Solyc08g061060.3.1.1	Downregulated	270	Solyc11g040140.2.1.1	Downregulated	330	Solyc05g008910.3.1.2	Downregulated
211	Solyc08g082980.2.1.7	Downregulated	271	Solyc11g066140.2.1.4	Downregulated	331	Solyc05g012660.3.1.2	Downregulated
212	Solyc08g081680.2.1.2	Downregulated	272	Solyc11g066950.2.1.1	Downregulated	332	Solyc01g067860.3.1.4	Downregulated
213	Solyc08g080750.4.1.3	Downregulated	273	Solyc11g069240.2.1.4	Downregulated	333	Solyc05g056400.3.1.8	Downregulated
214	Solyc08g078930.1.1.1	Downregulated	274	Solyc11g071470.1.1.1	Downregulated	334	Solyc05g051180.3.1.1	Downregulated
215	Solyc08g078910.1.1.1	Downregulated	275	Solyc11g069680.1.1.1	Downregulated	335	Solyc06g009280.1.1.5	Downregulated
216	Solyc08g078870.3.1.1	Downregulated	276	Solyc11g072770.2.1.1	Downregulated	336	Solyc04g008135.1.1.3	Downregulated
217	Solyc08g074980.4.1.1	Downregulated	277	Solyc12g005310.2.1.2	Downregulated	337	Solyc06g059990.4.1.1	Downregulated
218	Solyc08g074260.3.1.2	Downregulated	278	Solyc12g011360.2.1.3	Downregulated	338	Solyc07g005110.4.1.1	Downregulated
219	Solyc08g082460.4.1.2	Downregulated	279	Solyc03g083996.1.1.2	Downregulated	339	Solyc07g007320.3.1.1	Downregulated
220	Solyc08g080310.2.1.1	Downregulated	280	Solyc12g013610.2.1.1	Downregulated	340	Solyc07g009430.1.1.1	Downregulated
221	Solyc08g078880.3.1.1	Downregulated	281	Solyc12g036470.2.1.1	Downregulated	341	Solyc07g055970.1.1.1	Downregulated
222	Solyc09g015350.4.1.1	Downregulated	282	Solyc12g040640.2.1.1	Downregulated	342	Solyc07g065110.1.1.1	Downregulated
223	Solyc09g014820.3.1.3	Downregulated	283	Solyc12g040860.2.1.2	Downregulated	343	Solyc08g005500.4.1.2	Downregulated
224	Solyc09g013150.4.1.6	Downregulated	284	Solyc12g049550.2.1.5	Downregulated	344	Solyc01g087630.2.1.2	Downregulated
225	Solyc09g011540.2.1.1	Downregulated	285	Solyc12g056730.1.1.1	Downregulated	345	Solyc08g036505.2.1.1	Downregulated
226	Solyc09g010200.4.1.3	Downregulated	286	Solyc12g056050.1.1.1	Downregulated	346	Solyc08g068410.3.1.3	Downregulated
227	Solyc09g009980.3.1.1	Downregulated	287	Solyc12g077360.1.1.1	Downregulated	347	Solyc08g068520.3.1.1	Downregulated
228	Solyc09g008913.1.1.2	Downregulated	288	Solyc12g077370.3.1.1	Downregulated	348	Solyc09g066260.4.1.2	Downregulated
229	Solyc09g007660.1.1.1	Downregulated	289	Solyc12g099580.2.1.4	Downregulated	349	Solyc09g091400.4.1.2	Downregulated
230	Solyc09g007650.3.1.1	Downregulated	290	Solyc12g098320.3.1.1	Downregulated	350	Solyc09g092235.1.1.1	Downregulated
231	Solyc09g005480.3.1.1	Downregulated	291	Solyc12g094610.3.1.5	Downregulated	351	Solyc10g024490.2.1.1	Downregulated
232	Solyc09g008910.2.1.1	Downregulated	292	Solyc10g011925.1.1.1	Downregulated	352	Solyc10g047170.2.1.13	Downregulated
233	Solyc09g025280.1.1.1	Downregulated	293	Solyc12g036267.1.1.1	Downregulated	353	Solyc10g076370.3.1.1	Downregulated
234	Solyc09g066450.3.1.1	Downregulated	294	Solyc05g054790.1.1.1	Downregulated	354	Solyc10g076400.2.1.1	Downregulated
235	Solyc09g075870.2.1.9	Downregulated	295	Solyc12g098607.1.1.1	Downregulated	355	Solyc10g009487.1.1.2	Downregulated
236	Solyc09g074590.1.1.1	Downregulated	296	Solyc06g150136.1.1.1	Downregulated	356	Solyc11g007520.1.1.1	Downregulated
237	Solyc09g073040.3.1.2	Downregulated	297	Solyc09g057900.3.1.4	Downregulated	357	Solyc01g105387.1.1.1	Downregulated
238	Solyc09g072700.4.1.1	Downregulated	298	Solyc11g150136.1.1.3	Downregulated	358	Solyc02g076630.1.1.1	Downregulated
239	Solyc09g082480.2.1.1	Downregulated	299	Solyc04g064650.1.1.1	Downregulated	359	Solyc02g082960.3.1.3	Downregulated
240	Solyc09g089990.3.1.1	Downregulated	300	Solyc01g110870.3.1.1	Downregulated	360	Solyc02g086480.1.1.1	Downregulated

S.No.	ID	Status	S.No.	ID	Status	S.No.	ID	Status
361	Solyc03g079880.4.1.1	Downregulated	401	Solyc02g062710.1.1.1	Downregulated	441	Solyc05g007620.1.1.1	Downregulated
362	Solyc03g116520.1.1.1	Downregulated	402	Solyc03g026270.3.1.1	Downregulated	442	Solyc05g018310.1.1.1	Downregulated
363	Solyc04g055130.1.1.1	Downregulated	403	Solyc03g020080.4.1.2	Downregulated	443	Solyc06g060210.1.1.1	Downregulated
364	Solyc04g078370.3.1.3	Downregulated	404	Solyc03g117260.2.1.2	Downregulated	444	Solyc06g060510.2.1.1	Downregulated
365	Solyc06g084170.3.1.3	Downregulated	405	Solyc01g108360.4.1.1	Downregulated	445	Solyc06g075520.3.1.4	Downregulated
366	Solyc06g061200.1.1.1	Downregulated	406	Solyc05g056410.1.1.1	Downregulated	446	Solyc08g060970.4.1.10	Downregulated
367	Solyc07g150147.1.1.1	Downregulated	407	Solyc06g082770.3.1.2	Downregulated	447	Solyc08g076620.1.1.1	Downregulated
368	Solyc07g009080.4.1.1	Downregulated	408	Solyc07g052150.4.1.1	Downregulated	448	Solyc08g005120.3.1.2	Downregulated
369	Solyc07g009050.3.1.1	Downregulated	409	Solyc07g063800.2.1.7	Downregulated	449	Solyc09g065750.3.1.1	Downregulated
370	Solyc01g106140.2.1.2	Downregulated	410	Solyc07g065330.3.1.2	Downregulated	450	Solyc09g092410.4.1.2	Downregulated
371	Solyc01g110770.2.1.3	Downregulated	411	Solyc07g055690.1.1.1	Downregulated	451	Solyc09g005430.3.1.2	Downregulated
372	Solyc01g110680.4.1.1	Downregulated	412	Solyc08g079930.2.1.1	Downregulated	452	Solyc07g062670.1.1.1	Downregulated
373	Solyc01g110670.3.1.1	Downregulated	413	Solyc08g079970.2.1.1	Downregulated	453	Solyc02g086950.1.1.1	Downregulated
374	Solyc08g067500.1.1.1	Downregulated	414	Solyc08g079900.3.1.1	Downregulated	454	Solyc05g018770.2.1.1	Downregulated
375	Solyc08g080530.1.1.1	Downregulated	415	Solyc08g078940.1.1.1	Downregulated	455	Solyc05g010270.1.1.1	Downregulated
376	Solyc09g009000.4.1.1	Downregulated	416	Solyc08g008430.4.1.2	Downregulated	456	Solyc01g090980.1.1.1	Downregulated
377	Solyc09g083090.4.1.1	Downregulated	417	Solyc09g091210.4.1.1	Downregulated	457	Solyc06g052020.2.1.1	Downregulated
378	Solyc10g008650.2.1.1	Downregulated	418	Solyc09g011120.1.1.1	Downregulated	458	Solyc03g122000.4.1.1	Downregulated
379	Solyc03g006730.3.1.1	Downregulated	419	Solyc09g005420.4.1.1	Downregulated			
380	Solyc03g006750.1.1.1	Downregulated	420	Solyc09g014870.1.1.1	Downregulated			
381	Solyc01g096620.4.1.2	Downregulated	421	Solyc01g094260.4.1.5	Downregulated			
382	Solyc10g086320.2.1.7	Downregulated	422	Solyc01g094270.3.1.5	Downregulated			
383	Solyc08g150139.1.1.1	Downregulated	423	Solyc06g083120.1.1.1	Downregulated			
384	Solyc07g008930.1.1.1	Downregulated	424	Solyc07g008980.3.1.2	Downregulated			
385	Solyc04g017740.1.1.1	Downregulated	425	Solyc10g017980.1.1.1	Downregulated			
386	Solyc02g088980.3.1.2	Downregulated	426	Solyc10g011930.1.1.1	Downregulated			
387	Solyc06g054290.3.1.1	Downregulated	427	Solyc11g018774.1.1.1	Downregulated			
388	Solyc01g101195.1.1.2	Downregulated	428	Solyc11g069560.2.1.1	Downregulated			
389	Solyc07g047827.1.1.2	Downregulated	429	Solyc12g017700.2.1.3	Downregulated			
390	Solyc01g100100.4.1.1	Downregulated	430	Solyc02g063527.1.1.7	Downregulated			
391	Solyc02g091260.1.1.1	Downregulated	431	Solyc01g073840.1.1.1	Downregulated			
392	Solyc01g110940.3.1.1	Downregulated	432	Solyc02g071700.3.1.3	Downregulated			
393	Solyc10g083410.1.1.1	Downregulated	433	Solyc03g096300.3.1.2	Downregulated			
394	Solyc12g088860.2.1.1	Downregulated	434	Solyc03g115710.1.1.1	Downregulated			
395	Solyc02g079720.1.1.1	Downregulated	435	Solyc03g005500.1.1.1	Downregulated			
396	Solyc09g097810.3.1.2	Downregulated	436	Solyc08g062250.3.1.1	Downregulated			
397	Solyc07g044970.1.1.1	Downregulated	437	Solyc04g011960.2.1.1	Downregulated			
398	Solyc01g097690.2.1.1	Downregulated	438	Solyc04g014220.1.1.1	Downregulated			
399	Solyc01g107810.2.1.1	Downregulated	439	Solyc04g055150.1.1.1	Downregulated			
400	Solyc01g110930.1.1.1	Downregulated	440	Solyc04g055140.1.1.1	Downregulated			

Annexure 7: Differentially expressed genes in RNA seq of CLN (Tolerant variety).

S.No.	id	Status	S.No.	id	Status	S.No.	id	Status
1	Solyc11g022590.1.1.1	Up regulated	66	Solyc01g101180.4.1.6	Up regulated	131	Solyc01g107760.4.1.2	Up regulated
2	Solyc02g092800.3.1.2	Up regulated	67	Solyc01g101190.3.1.6	Up regulated	132	Solyc01g108350.4.1.1	Up regulated
3	Solyc03g119770.4.1.1	Up regulated	68	Solyc09g091000.4.1.2	Up regulated	133	Solyc01g108880.4.1.3	Up regulated
4	Solyc01g006540.4.1.10	Up regulated	69	Solyc07g006380.3.1.2	Up regulated	134	Solyc01g109120.3.1.1	Up regulated
5	Solyc07g049460.3.1.5	Up regulated	70	Solyc10g075050.2.1.2	Up regulated	135	Solyc01g110110.3.1.1	Up regulated
6	Solyc08g068610.3.1.4	Up regulated	71	Solyc12g006680.2.1.1	Up regulated	136	Solyc01g109390.3.1.1	Up regulated
7	Solyc12g010030.3.1.1	Up regulated	72	Solyc11g066700.2.1.1	Up regulated	137	Solyc01g112230.4.1.1	Up regulated
8	Solyc08g078700.2.1.2	Up regulated	73	Solyc02g083860.3.1.2	Up regulated	138	Solyc01g087280.2.1.9	Up regulated
9	Solyc09g061280.4.1.3	Up regulated	74	Solyc04g076845.1.1.1	Up regulated	139	Solyc01g088790.2.1.3	Up regulated
10	Solyc02g032910.3.1.1	Up regulated	75	Solyc03g111730.3.1.4	Up regulated	140	Solyc01g095960.4.1.2	Up regulated
11	Solyc09g091510.3.1.2	Up regulated	76	Solyc04g078460.3.1.1	Up regulated	141	Solyc01g105350.3.1.2	Up regulated
12	Solyc05g053550.3.1.2	Up regulated	77	Solyc06g051940.4.1.3	Up regulated	142	Solyc01g105610.1.1.1	Up regulated
13	Solyc07g026650.3.1.1	Up regulated	78	Solyc02g085910.4.1.1	Up regulated	143	Solyc01g105770.2.1.1	Up regulated
14	Solyc02g084850.3.1.1	Up regulated	79	Solyc03g098100.4.1.2	Up regulated	144	Solyc01g110140.4.1.2	Up regulated
15	Solyc03g006880.3.1.1	Up regulated	80	Solyc10g085880.1.1.1	Up regulated	145	Solyc02g014730.3.1.2	Up regulated
16	Solyc04g076880.3.1.13	Up regulated	81	Solyc10g085890.1.1.1	Up regulated	146	Solyc02g032820.3.1.1	Up regulated
17	Solyc09g090970.4.1.1	Up regulated	82	Solyc04g005610.3.1.3	Up regulated	147	Solyc02g093700.3.1.1	Up regulated
18	Solyc03g098790.3.1.1	Up regulated	83	Solyc03g044180.1.1.1	Up regulated	148	Solyc02g093180.3.1.1	Up regulated
19	Solyc09g089610.3.1.2	Up regulated	84	Solyc05g054900.3.1.2	Up regulated	149	Solyc02g092820.4.1.1	Up regulated
20	Solyc08g008110.2.1.1	Up regulated	85	Solyc08g016720.1.1.1	Up regulated	150	Solyc02g091070.3.1.6	Up regulated
21	Solyc01g095080.3.1.1	Up regulated	86	Solyc01g008670.4.1.1	Up regulated	151	Solyc02g090970.1.1.1	Up regulated
22	Solyc01g068410.4.1.1	Up regulated	87	Solyc01g150101.1.1.6	Up regulated	152	Solyc02g090210.3.1.5	Up regulated
23	Solyc06g063330.3.1.1	Up regulated	88	Solyc01g006930.3.1.6	Up regulated	153	Solyc02g089140.3.1.3	Up regulated
24	Solyc09g098160.3.1.6	Up regulated	89	Solyc01g005500.3.1.3	Up regulated	154	Solyc02g086530.4.1.1	Up regulated
25	Solyc11g018530.2.1.1	Up regulated	90	Solyc01g006050.2.1.1	Up regulated	155	Solyc02g085630.3.1.2	Up regulated
26	Solyc02g083520.2.1.1	Up regulated	91	Solyc01g009370.2.1.1	Up regulated	156	Solyc02g084950.3.1.4	Up regulated
27	Solyc06g036260.3.1.7	Up regulated	92	Solyc01g006530.1.1.1	Up regulated	157	Solyc02g082910.4.1.1	Up regulated
28	Solyc03g115890.3.1.3	Up regulated	93	Solyc01g060260.4.1.4	Up regulated	158	Solyc02g082850.3.1.10	Up regulated
29	Solyc11g065600.2.1.1	Up regulated	94	Solyc01g057910.3.1.3	Up regulated	159	Solyc02g082450.3.1.6	Up regulated
30	Solyc07g056570.1.1.1	Up regulated	95	Solyc01g058250.2.1.2	Up regulated	160	Solyc02g080850.1.1.1	Up regulated
31	Solyc04g078900.3.1.1	Up regulated	96	Solyc01g067295.1.1.1	Up regulated	161	Solyc02g079520.3.1.1	Up regulated
32	Solyc01g109140.3.1.1	Up regulated	97	Solyc01g066570.3.1.2	Up regulated	162	Solyc02g079430.4.1.2	Up regulated
33	Solyc04g082030.1.1.1	Up regulated	98	Solyc01g065740.3.1.4	Up regulated	163	Solyc02g078250.4.1.1	Up regulated
34	Solyc03g098795.1.1.2	Up regulated	99	Solyc01g079300.4.1.4	Up regulated	164	Solyc02g077020.3.1.1	Up regulated
35	Solyc08g005670.2.1.2	Up regulated	100	Solyc01g080410.3.1.3	Up regulated	165	Solyc02g071710.3.1.5	Up regulated
36	Solyc08g005680.4.1.4	Up regulated	101	Solyc01g080870.3.1.1	Up regulated	166	Solyc02g069730.3.1.1	Up regulated
37	Solyc03g096670.3.1.1	Up regulated	102	Solyc01g081250.3.1.3	Up regulated	167	Solyc02g069510.1.1.1	Up regulated
38	Solyc06g059930.4.1.2	Up regulated	103	Solyc01g081590.4.1.2	Up regulated	168	Solyc02g068380.3.1.4	Up regulated
39	Solyc09g089930.3.1.1	Up regulated	104	Solyc01g081600.3.1.2	Up regulated	169	Solyc02g067680.1.1.1	Up regulated
40	Solyc02g086880.4.1.3	Up regulated	105	Solyc01g079670.2.1.1	Up regulated	170	Solyc02g067440.3.1.1	Up regulated
41	Solyc12g056600.3.1.2	Up regulated	106	Solyc01g087180.4.1.1	Up regulated	171	Solyc02g065430.1.1.1	Up regulated
42	Solyc01g101170.4.1.1	Up regulated	107	Solyc01g088430.4.1.10	Up regulated	172	Solyc02g065240.3.1.1	Up regulated
43	Solyc01g088160.4.1.5	Up regulated	108	Solyc01g090340.3.1.1	Up regulated	173	Solyc02g065050.1.1.1	Up regulated
44	Solyc10g086250.2.1.1	Up regulated	109	Solyc01g090730.3.1.1	Up regulated	174	Solyc02g062500.3.1.3	Up regulated
45	Solyc06g084070.3.1.1	Up regulated	110	Solyc01g090790.4.1.5	Up regulated	175	Solyc02g062460.4.1.3	Up regulated
46	Solyc02g061770.4.1.3	Up regulated	111	Solyc01g090890.3.1.3	Up regulated	176	Solyc02g062390.3.1.2	Up regulated
47	Solyc06g062540.3.1.4	Up regulated	112	Solyc01g096280.2.1.2	Up regulated	177	Solyc02g094270.2.1.2	Up regulated
48	Solyc07g064600.3.1.6	Up regulated	113	Solyc01g096320.3.1.2	Up regulated	178	Solyc02g094040.3.1.9	Up regulated
49	Solyc08g066650.3.1.5	Up regulated	114	Solyc01g096720.4.1.8	Up regulated	179	Solyc02g090980.1.1.1	Up regulated
50	Solyc04g054730.3.1.12	Up regulated	115	Solyc01g097470.4.1.1	Up regulated	180	Solyc02g086850.3.1.7	Up regulated
51	Solyc03g115220.4.1.1	Up regulated	116	Solyc01g098720.3.1.2	Up regulated	181	Solyc02g085150.4.1.3	Up regulated
52	Solyc01g100370.3.1.1	Up regulated	117	Solyc01g099620.3.1.2	Up regulated	182	Solyc02g084910.4.1.2	Up regulated
53	Solyc02g038740.4.1.4	Up regulated	118	Solyc01g099660.4.1.7	Up regulated	183	Solyc02g080840.1.1.1	Up regulated
54	Solyc09g089580.4.1.1	Up regulated	119	Solyc01g099880.4.1.1	Up regulated	184	Solyc02g079150.2.1.3	Up regulated
55	Solyc09g092520.3.1.1	Up regulated	120	Solyc01g102260.3.1.1	Up regulated	185	Solyc02g077580.1.1.1	Up regulated
56	Solyc05g051850.3.1.4	Up regulated	121	Solyc01g102890.4.1.1	Up regulated	186	Solyc02g077360.1.1.1	Up regulated
57	Solyc12g014360.2.1.5	Up regulated	122	Solyc01g102910.4.1.2	Up regulated	187	Solyc02g070610.3.1.1	Up regulated
58	Solyc07g055920.4.1.2	Up regulated	123	Solyc01g102960.3.1.1	Up regulated	188	Solyc02g070110.1.1.1	Up regulated
59	Solyc11g011340.2.1.3	Up regulated	124	Solyc01g105370.4.1.1	Up regulated	189	Solyc03g005210.4.1.1	Up regulated
60	Solyc01g057000.3.1.1	Up regulated	125	Solyc01g106060.3.1.4	Up regulated	190	Solyc03g007360.3.1.2	Up regulated
61	Solyc02g086310.2.1.1	Up regulated	126	Solyc01g105000.3.1.1	Up regulated	191	Solyc03g007690.1.1.1	Up regulated
62	Solyc03g111720.3.1.1	Up regulated	127	Solyc01g106600.2.1.1	Up regulated	192	Solyc03g005520.1.1.1	Up regulated
63	Solyc09g082690.3.1.3	Up regulated	128	Solyc01g106920.4.1.1	Up regulated	193	Solyc03g006900.2.1.4	Up regulated
64	Solyc02g077290.2.1.2	Up regulated	129	Solyc01g107080.3.1.1	Up regulated	194	Solyc03g019690.1.1.1	Up regulated
65	Solyc01g101210.4.1.6	Up regulated	130	Solyc01g107460.2.1.1	Up regulated	195	Solyc03g025720.3.1.2	Up regulated

S.No.	id	Status	S.No.	id	Status	S.No.	id	Status
196	Solyc03g025810.4.1.2	Up regulated	261	Solyc04g080970.4.1.2	Up regulated	326	Solyc06g061210.4.1.3	Up regulated
197	Solyc03g031630.3.1.2	Up regulated	262	Solyc04g072300.1.1.1	Up regulated	327	Solyc06g060910.2.1.3	Up regulated
198	Solyc03g032170.2.1.1	Up regulated	263	Solyc04g080500.3.1.2	Up regulated	328	Solyc06g083850.4.1.2	Up regulated
199	Solyc03g034030.3.1.4	Up regulated	264	Solyc04g080990.2.1.1	Up regulated	329	Solyc06g082430.2.1.1	Up regulated
200	Solyc03g083500.3.1.2	Up regulated	265	Solyc04g081120.1.1.1	Up regulated	330	Solyc06g082420.4.1.4	Up regulated
201	Solyc03g078770.3.1.2	Up regulated	266	Solyc05g005170.4.1.7	Up regulated	331	Solyc06g082240.2.1.5	Up regulated
202	Solyc03g078500.3.1.2	Up regulated	267	Solyc05g005280.4.1.4	Up regulated	332	Solyc07g005370.4.1.1	Up regulated
203	Solyc03g078490.4.1.2	Up regulated	268	Solyc05g009120.3.1.1	Up regulated	333	Solyc07g005820.3.1.1	Up regulated
204	Solyc03g094020.3.1.8	Up regulated	269	Solyc05g009500.3.1.1	Up regulated	334	Solyc07g006680.1.1.1	Up regulated
205	Solyc03g096540.3.1.1	Up regulated	270	Solyc05g010330.4.1.4	Up regulated	335	Solyc07g006860.3.1.3	Up regulated
206	Solyc03g096545.1.1.1	Up regulated	271	Solyc05g010516.1.1.1	Up regulated	336	Solyc07g008210.4.1.3	Up regulated
207	Solyc03g096770.1.1.1	Up regulated	272	Solyc05g012955.1.1.2	Up regulated	337	Solyc07g008360.2.1.1	Up regulated
208	Solyc03g096780.1.1.1	Up regulated	273	Solyc05g013010.3.1.2	Up regulated	338	Solyc07g008710.3.1.2	Up regulated
209	Solyc03g097500.3.1.3	Up regulated	274	Solyc05g016690.3.1.1	Up regulated	339	Solyc07g017980.1.1.1	Up regulated
210	Solyc03g097440.3.1.6	Up regulated	275	Solyc05g018880.1.1.1	Up regulated	340	Solyc07g056510.3.1.2	Up regulated
211	Solyc03g098670.1.1.1	Up regulated	276	Solyc05g024260.3.1.6	Up regulated	341	Solyc07g055560.4.1.5	Up regulated
212	Solyc03g098700.1.1.1	Up regulated	277	Solyc05g006670.2.1.1	Up regulated	342	Solyc07g054790.1.1.1	Up regulated
213	Solyc03g093620.1.1.1	Up regulated	278	Solyc05g056470.1.1.1	Up regulated	343	Solyc07g054780.1.1.1	Up regulated
214	Solyc03g098710.1.1.1	Up regulated	279	Solyc05g053620.3.1.3	Up regulated	344	Solyc07g054760.1.1.1	Up regulated
215	Solyc03g098740.1.1.1	Up regulated	280	Solyc05g052680.1.1.1	Up regulated	345	Solyc07g053140.3.1.1	Up regulated
216	Solyc03g123680.1.1.1	Up regulated	281	Solyc05g052670.1.1.1	Up regulated	346	Solyc07g052370.4.1.1	Up regulated
217	Solyc03g122140.3.1.1	Up regulated	282	Solyc05g052370.3.1.1	Up regulated	347	Solyc07g051950.3.1.2	Up regulated
218	Solyc03g120870.3.1.3	Up regulated	283	Solyc05g051940.3.1.2	Up regulated	348	Solyc07g042490.1.1.1	Up regulated
219	Solyc07g014600.2.1.2	Up regulated	284	Solyc05g051550.2.1.3	Up regulated	349	Solyc07g042470.3.1.1	Up regulated
220	Solyc03g119840.3.1.4	Up regulated	285	Solyc05g051210.3.1.2	Up regulated	350	Solyc07g054950.2.1.5	Up regulated
221	Solyc03g118190.4.1.1	Up regulated	286	Solyc05g050210.4.1.1	Up regulated	351	Solyc07g052620.1.1.1	Up regulated
222	Solyc03g116610.3.1.2	Up regulated	287	Solyc05g047680.4.1.2	Up regulated	352	Solyc07g049600.4.1.1	Up regulated
223	Solyc03g115960.3.1.1	Up regulated	288	Solyc05g047530.3.1.2	Up regulated	353	Solyc07g042430.1.1.4	Up regulated
224	Solyc03g116530.3.1.1	Up regulated	289	Solyc05g046140.3.1.1	Up regulated	354	Solyc07g064990.3.1.3	Up regulated
225	Solyc03g113540.3.1.1	Up regulated	290	Solyc05g039950.2.1.1	Up regulated	355	Solyc07g061800.4.1.1	Up regulated
226	Solyc03g112340.1.1.1	Up regulated	291	Solyc05g053890.3.1.1	Up regulated	356	Solyc08g006020.4.1.3	Up regulated
227	Solyc03g112040.1.1.1	Up regulated	292	Solyc05g053150.2.1.1	Up regulated	357	Solyc08g006150.3.1.8	Up regulated
228	Solyc03g111860.1.1.1	Up regulated	293	Solyc06g007970.3.1.1	Up regulated	358	Solyc08g007270.4.1.1	Up regulated
229	Solyc03g111815.1.1.1	Up regulated	294	Solyc06g008368.1.1.3	Up regulated	359	Solyc08g008240.3.1.1	Up regulated
230	Solyc03g111510.3.1.5	Up regulated	295	Solyc06g009050.3.1.1	Up regulated	360	Solyc08g008360.2.1.1	Up regulated
231	Solyc03g111400.1.1.1	Up regulated	296	Solyc06g035960.3.1.3	Up regulated	361	Solyc08g013740.4.1.6	Up regulated
232	Solyc03g111100.1.1.1	Up regulated	297	Solyc06g035710.1.1.1	Up regulated	362	Solyc08g014010.4.1.10	Up regulated
233	Solyc03g121040.4.1.1	Up regulated	298	Solyc06g034410.3.1.1	Up regulated	363	Solyc08g014560.3.1.1	Up regulated
234	Solyc03g115310.1.1.1	Up regulated	299	Solyc06g050530.3.1.2	Up regulated	364	Solyc08g023510.3.1.1	Up regulated
235	Solyc03g113690.1.1.1	Up regulated	300	Solyc06g050900.3.1.4	Up regulated	365	Solyc08g036570.1.1.1	Up regulated
236	Solyc04g011600.4.1.2	Up regulated	301	Solyc06g051860.3.1.1	Up regulated	366	Solyc08g048290.4.1.2	Up regulated
237	Solyc04g010330.3.1.3	Up regulated	302	Solyc06g051840.1.1.1	Up regulated	367	Solyc08g062970.1.1.1	Up regulated
238	Solyc04g010320.1.1.1	Up regulated	303	Solyc06g054030.2.1.3	Up regulated	368	Solyc12g089385.1.1.1	Up regulated
239	Solyc04g008230.3.1.1	Up regulated	304	Solyc06g076800.3.1.2	Up regulated	369	Solyc08g066100.3.1.2	Up regulated
240	Solyc04g007825.2.1.2	Up regulated	305	Solyc06g076760.2.1.5	Up regulated	370	Solyc08g067160.3.1.10	Up regulated
241	Solyc04g005100.3.1.3	Up regulated	306	Solyc06g076400.3.1.1	Up regulated	371	Solyc08g068630.4.1.1	Up regulated
242	Solyc04g011940.3.1.1	Up regulated	307	Solyc06g076160.4.1.1	Up regulated	372	Solyc08g068665.1.1.1	Up regulated
243	Solyc04g005380.3.1.1	Up regulated	308	Solyc06g075480.4.1.1	Up regulated	373	Solyc08g068720.1.1.1	Up regulated
244	Solyc04g005300.4.1.1	Up regulated	309	Solyc06g074750.1.1.1	Up regulated	374	Solyc08g059700.2.1.4	Up regulated
245	Solyc04g083140.2.1.2	Up regulated	310	Solyc06g074140.1.1.1	Up regulated	375	Solyc08g081820.4.1.3	Up regulated
246	Solyc04g050790.3.1.1	Up regulated	311	Solyc06g073760.3.1.10	Up regulated	376	Solyc08g081630.2.1.1	Up regulated
247	Solyc04g064630.3.1.1	Up regulated	312	Solyc06g072990.1.1.1	Up regulated	377	Solyc08g081350.2.1.4	Up regulated
248	Solyc04g064770.1.1.2	Up regulated	313	Solyc06g072870.1.1.1	Up regulated	378	Solyc08g079420.3.1.1	Up regulated
249	Solyc04g071340.5.1.12	Up regulated	314	Solyc06g071820.3.1.2	Up regulated	379	Solyc08g078460.3.1.7	Up regulated
250	Solyc04g071520.3.1.7	Up regulated	315	Solyc06g068130.3.1.1	Up regulated	380	Solyc08g078180.1.1.1	Up regulated
251	Solyc04g071600.3.1.1	Up regulated	316	Solyc06g067980.3.1.3	Up regulated	381	Solyc08g077910.3.1.3	Up regulated
252	Solyc04g071780.3.1.2	Up regulated	317	Solyc06g066750.3.1.2	Up regulated	382	Solyc08g077900.3.1.3	Up regulated
253	Solyc04g072280.3.1.5	Up regulated	318	Solyc06g062490.3.1.1	Up regulated	383	Solyc08g077330.3.1.1	Up regulated
254	Solyc04g072760.3.1.1	Up regulated	319	Solyc06g062460.3.1.1	Up regulated	384	Solyc08g076250.3.1.1	Up regulated
255	Solyc04g074990.3.1.3	Up regulated	320	Solyc06g062370.4.1.3	Up regulated	385	Solyc08g075880.4.1.1	Up regulated
256	Solyc04g077170.3.1.1	Up regulated	321	Solyc06g060970.2.1.2	Up regulated	386	Solyc08g075320.4.1.5	Up regulated
257	Solyc04g077740.1.1.1	Up regulated	322	Solyc06g074640.2.1.9	Up regulated	387	Solyc08g079800.4.1.1	Up regulated
258	Solyc04g078660.2.1.1	Up regulated	323	Solyc06g066230.4.1.2	Up regulated	388	Solyc08g078620.1.1.1	Up regulated
259	Solyc04g079260.3.1.3	Up regulated	324	Solyc06g065420.2.1.3	Up regulated	389	Solyc08g076710.3.1.1	Up regulated
260	Solyc04g080540.2.1.4	Up regulated	325	Solyc06g063280.1.1.1	Up regulated	390	Solyc08g076700.1.1.1	Up regulated

S.No.	id	Status	S.No.	id	Status	S.No.	id	Status
391	Solyc09g014930.1.1.1	Up regulated	456	Solyc10g078780.2.1.1	Up regulated	521	Solyc10g018140.2.1.1	Up regulated
392	Solyc09g014750.1.1.1	Up regulated	457	Solyc10g079380.2.1.2	Up regulated	522	Solyc06g036190.1.1.1	Up regulated
393	Solyc09g011550.3.1.1	Up regulated	458	Solyc11g006300.2.1.1	Up regulated	523	Solyc02g079800.1.1.1	Up regulated
394	Solyc09g011520.3.1.1	Up regulated	459	Solyc11g006490.2.1.1	Up regulated	524	Solyc01g011105.1.1.1	Up regulated
395	Solyc09g008780.4.1.12	Up regulated	460	Solyc11g007390.1.1.1	Up regulated	525	Solyc01g101260.2.1.1	Up regulated
396	Solyc09g005970.1.1.1	Up regulated	461	Solyc11g007500.2.1.1	Up regulated	526	Solyc05g025985.1.1.1	Up regulated
397	Solyc09g005730.4.1.3	Up regulated	462	Solyc11g010740.3.1.1	Up regulated	527	Solyc01g150126.1.1.2	Up regulated
398	Solyc09g005210.4.1.2	Up regulated	463	Solyc11g011110.3.1.1	Up regulated	528	Solyc01g100070.3.1.1	Up regulated
399	Solyc09g011350.3.1.1	Up regulated	464	Solyc11g011330.2.1.3	Up regulated	529	Solyc02g092890.1.1.1	Up regulated
400	Solyc09g010650.1.1.1	Up regulated	465	Solyc11g012120.2.1.2	Up regulated	530	Solyc02g068460.1.1.1	Up regulated
401	Solyc09g008770.3.1.1	Up regulated	466	Solyc11g013110.2.1.1	Up regulated	531	Solyc05g010000.1.1.1	Up regulated
402	Solyc09g061700.3.1.1	Up regulated	467	Solyc11g005310.1.1.1	Up regulated	532	Solyc02g090120.1.1.1	Up regulated
403	Solyc09g064160.3.1.3	Up regulated	468	Solyc11g005860.2.1.5	Up regulated	533	Solyc01g006640.1.1.6	Up regulated
404	Solyc09g064820.1.1.1	Up regulated	469	Solyc11g006310.2.1.5	Up regulated	534	Solyc01g009270.1.1.1	Up regulated
405	Solyc09g065410.4.1.3	Up regulated	470	Solyc11g007490.1.1.1	Up regulated	535	Solyc10g079390.1.1.1	Up regulated
406	Solyc09g065440.4.1.1	Up regulated	471	Solyc11g013220.2.1.1	Up regulated	536	Solyc11g073075.1.1.1	Up regulated
407	Solyc09g065420.3.1.3	Up regulated	472	Solyc11g040140.2.1.1	Up regulated	537	Solyc12g088190.2.1.7	Up regulated
408	Solyc09g066150.2.1.1	Up regulated	473	Solyc11g065520.1.1.1	Up regulated	538	Solyc12g099130.2.1.3	Up regulated
409	Solyc09g066410.3.1.1	Up regulated	474	Solyc11g069240.2.1.4	Up regulated	539	Solyc01g008420.3.1.4	Up regulated
410	Solyc09g066350.1.1.1	Up regulated	475	Solyc11g069640.2.1.6	Up regulated	540	Solyc01g107090.2.1.1	Up regulated
411	Solyc09g074760.1.1.1	Up regulated	476	Solyc11g071480.1.1.1	Up regulated	541	Solyc01g150131.1.1.3	Up regulated
412	Solyc09g074570.1.1.1	Up regulated	477	Solyc11g071940.1.1.1	Up regulated	542	Solyc02g077970.4.1.1	Up regulated
413	Solyc09g075700.1.1.1	Up regulated	478	Solyc11g072380.2.1.1	Up regulated	543	Solyc02g072070.4.1.1	Up regulated
414	Solyc09g075400.4.1.1	Up regulated	479	Solyc11g072470.3.1.1	Up regulated	544	Solyc02g070660.3.1.5	Up regulated
415	Solyc09g082270.4.1.3	Up regulated	480	Solyc11g073120.2.1.3	Up regulated	545	Solyc02g065020.2.1.1	Up regulated
416	Solyc09g082280.3.1.3	Up regulated	481	Solyc12g005450.1.1.1	Up regulated	546	Solyc02g062380.3.1.1	Up regulated
417	Solyc09g082460.3.1.5	Up regulated	482	Solyc12g010920.2.1.1	Up regulated	547	Solyc02g061990.3.1.1	Up regulated
418	Solyc09g082550.3.1.12	Up regulated	483	Solyc12g010960.2.1.1	Up regulated	548	Solyc02g072570.2.1.1	Up regulated
419	Solyc09g083435.1.1.3	Up regulated	484	Solyc03g083996.1.1.2	Up regulated	549	Solyc03g007230.4.1.1	Up regulated
420	Solyc09g084490.4.1.3	Up regulated	485	Solyc12g017340.1.1.1	Up regulated	550	Solyc03g007720.3.1.2	Up regulated
421	Solyc09g089540.4.1.2	Up regulated	486	Solyc12g019230.2.1.1	Up regulated	551	Solyc03g013440.4.1.2	Up regulated
422	Solyc09g089730.3.1.1	Up regulated	487	Solyc12g005440.1.1.1	Up regulated	552	Solyc03g026370.1.1.1	Up regulated
423	Solyc09g089860.4.1.7	Up regulated	488	Solyc12g013610.2.1.1	Up regulated	553	Solyc03g071750.3.1.1	Up regulated
424	Solyc09g090300.3.1.2	Up regulated	489	Solyc12g035400.2.1.4	Up regulated	554	Solyc03g094120.3.1.1	Up regulated
425	Solyc09g090500.3.1.14	Up regulated	490	Solyc12g036470.2.1.1	Up regulated	555	Solyc03g095700.1.1.1	Up regulated
426	Solyc09g090790.3.1.1	Up regulated	491	Solyc12g036480.2.1.1	Up regulated	556	Solyc03g120020.4.1.13	Up regulated
427	Solyc09g091530.2.1.4	Up regulated	492	Solyc12g056610.3.1.3	Up regulated	557	Solyc03g113850.1.1.1	Up regulated
428	Solyc09g091670.3.1.17	Up regulated	493	Solyc12g055710.1.1.1	Up regulated	558	Solyc01g059940.2.1.1	Up regulated
429	Solyc09g092490.3.1.2	Up regulated	494	Solyc12g056730.1.1.1	Up regulated	559	Solyc04g018090.2.1.2	Up regulated
430	Solyc09g092560.4.1.3	Up regulated	495	Solyc12g088640.2.1.2	Up regulated	560	Solyc04g009520.3.1.4	Up regulated
431	Solyc09g092580.4.1.2	Up regulated	496	Solyc12g088970.1.1.3	Up regulated	561	Solyc04g054150.2.1.1	Up regulated
432	Solyc09g092620.3.1.2	Up regulated	497	Solyc12g088180.1.1.1	Up regulated	562	Solyc04g082830.3.1.9	Up regulated
433	Solyc09g089910.1.1.1	Up regulated	498	Solyc12g100270.2.1.5	Up regulated	563	Solyc05g005610.4.1.1	Up regulated
434	Solyc09g098080.4.1.1	Up regulated	499	Solyc12g100250.3.1.1	Up regulated	564	Solyc05g012660.3.1.2	Up regulated
435	Solyc10g009270.3.1.1	Up regulated	500	Solyc12g098900.2.1.2	Up regulated	565	Solyc01g067620.3.1.3	Up regulated
436	Solyc10g008330.4.1.1	Up regulated	501	Solyc12g098590.3.1.2	Up regulated	566	Solyc05g052410.3.1.1	Up regulated
437	Solyc02g061620.2.1.2	Up regulated	502	Solyc12g096310.2.1.2	Up regulated	567	Solyc05g052240.3.1.4	Up regulated
438	Solyc10g008040.4.1.1	Up regulated	503	Solyc12g094690.1.1.1	Up regulated	568	Solyc05g050220.3.1.12	Up regulated
439	Solyc10g005090.3.1.1	Up regulated	504	Solyc04g072037.1.1.1	Up regulated	569	Solyc01g079130.2.1.6	Up regulated
440	Solyc10g055560.2.1.2	Up regulated	505	Solyc04g076730.1.1.1	Up regulated	570	Solyc04g056713.1.1.1	Up regulated
441	Solyc10g052500.2.1.1	Up regulated	506	Solyc05g018930.1.1.1	Up regulated	571	Solyc07g006370.1.1.1	Up regulated
442	Solyc10g050880.2.1.1	Up regulated	507	Solyc03g119550.3.1.5	Up regulated	572	Solyc07g008520.3.1.6	Up regulated
443	Solyc10g048030.2.1.1	Up regulated	508	Solyc08g021880.1.1.1	Up regulated	573	Solyc07g008570.3.1.11	Up regulated
444	Solyc10g075170.3.1.6	Up regulated	509	Solyc08g081880.3.1.2	Up regulated	574	Solyc07g054770.1.1.1	Up regulated
445	Solyc10g076250.2.1.1	Up regulated	510	Solyc03g112090.3.1.1	Up regulated	575	Solyc07g051930.3.1.1	Up regulated
446	Solyc10g076510.2.1.5	Up regulated	511	Solyc04g064470.2.1.1	Up regulated	576	Solyc07g044920.1.1.1	Up regulated
447	Solyc10g078230.3.1.1	Up regulated	512	Solyc07g064750.1.1.2	Up regulated	577	Solyc07g064660.1.1.1	Up regulated
448	Solyc10g078270.2.1.1	Up regulated	513	Solyc07g064650.3.1.1	Up regulated	578	Solyc08g014190.4.1.1	Up regulated
449	Solyc10g081560.3.1.1	Up regulated	514	Solyc12g150105.1.1.2	Up regulated	579	Solyc08g078550.1.1.1	Up regulated
450	Solyc10g083440.2.1.1	Up regulated	515	Solyc07g062460.4.1.5	Up regulated	580	Solyc08g078170.1.1.1	Up regulated
451	Solyc10g083690.3.1.1	Up regulated	516	Solyc12g006130.3.1.1	Up regulated	581	Solyc08g075940.4.1.3	Up regulated
452	Solyc10g084890.3.1.1	Up regulated	517	Solyc04g077300.3.1.2	Up regulated	582	Solyc01g091250.3.1.1	Up regulated
453	Solyc10g084900.1.1.1	Up regulated	518	Solyc05g006610.4.1.1	Up regulated	583	Solyc09g014910.4.1.2	Up regulated
454	Solyc10g085870.1.1.1	Up regulated	519	Solyc07g063640.1.1.1	Up regulated	584	Solyc09g007470.2.1.1	Up regulated
455	Solyc10g086270.2.1.1	Up regulated	520	Solyc01g008870.1.1.1	Up regulated	585	Solyc09g065430.4.1.3	Up regulated

S.No.	id	Status	S.No.	id	Status	S.No.	id	Status
586	Solyc09g075270.1.1.1	Up regulated	651	Solyc02g067730.2.1.1	Up regulated	716	Solyc06g076310.1.1.1	Up regulated
587	Solyc09g082300.3.1.1	Up regulated	652	Solyc03g116190.2.1.1	Up regulated			
588	Solyc09g091400.4.1.2	Up regulated	653	Solyc03g007340.4.1.1	Up regulated			
589	Solyc09g091960.3.1.1	Up regulated	654	Solyc09g066190.1.1.1	Up regulated			
590	Solyc09g092725.1.1.2	Up regulated	655	Solyc11g069770.2.1.1	Up regulated			
591	Solyc10g024490.2.1.1	Up regulated	656	Solyc12g005380.3.1.14	Up regulated			
592	Solyc10g055620.2.1.14	Up regulated	657	Solyc12g098110.1.1.1	Up regulated			
593	Solyc02g087890.4.1.2	Up regulated	658	Solyc12g100260.1.1.1	Up regulated			
594	Solyc10g007870.3.1.1	Up regulated	659	Solyc02g093890.1.1.1	Up regulated			
595	Solyc10g074390.1.1.1	Up regulated	660	Solyc02g065480.2.1.1	Up regulated			
596	Solyc10g078770.2.1.1	Up regulated	661	Solyc01g105767.1.1.1	Up regulated			
597	Solyc10g081780.3.1.1	Up regulated	662	Solyc03g097570.3.1.1	Up regulated			
598	Solyc11g065990.2.1.2	Up regulated	663	Solyc03g112010.4.1.2	Up regulated			
599	Solyc01g112130.4.1.1	Up regulated	664	Solyc03g112030.3.1.1	Up regulated			
600	Solyc12g150125.1.1.2	Up regulated	665	Solyc01g067130.4.1.1	Up regulated			
601	Solyc12g056675.1.1.2	Up regulated	666	Solyc01g006620.3.1.1	Up regulated			
602	Solyc12g056678.1.1.1	Up regulated	667	Solyc01g006650.2.1.3	Up regulated			
603	Solyc12g056674.1.1.2	Up regulated	668	Solyc06g069150.1.1.1	Up regulated			
604	Solyc12g044954.1.1.2	Up regulated	669	Solyc06g069350.3.1.1	Up regulated			
605	Solyc01g107825.1.1.1	Up regulated	670	Solyc07g007770.2.1.2	Up regulated			
606	Solyc02g071430.3.1.4	Up regulated	671	Solyc07g007610.2.1.5	Up regulated			
607	Solyc02g063000.4.1.1	Up regulated	672	Solyc07g052120.4.1.1	Up regulated			
608	Solyc02g084940.1.1.1	Up regulated	673	Solyc07g063800.2.1.7	Up regulated			
609	Solyc02g084430.3.1.2	Up regulated	674	Solyc08g061890.4.1.1	Up regulated			
610	Solyc01g086680.4.1.2	Up regulated	675	Solyc09g065740.2.1.4	Up regulated			
611	Solyc03g096190.2.1.1	Up regulated	676	Solyc01g094240.3.1.5	Up regulated			
612	Solyc04g007810.1.1.1	Up regulated	677	Solyc10g081840.3.1.5	Up regulated			
613	Solyc04g071590.3.1.2	Up regulated	678	Solyc05g010170.2.1.1	Up regulated			
614	Solyc05g015850.4.1.2	Up regulated	679	Solyc06g060675.1.1.1	Up regulated			
615	Solyc06g068600.3.1.1	Up regulated	680	Solyc10g084400.2.1.1	Up regulated			
616	Solyc06g005660.3.1.1	Up regulated	681	Solyc11g068880.2.1.1	Up regulated			
617	Solyc06g064560.4.1.1	Up regulated	682	Solyc12g008440.1.1.1	Up regulated			
618	Solyc06g083290.1.1.1	Up regulated	683	Solyc01g091170.3.1.7	Up regulated			
619	Solyc01g059950.1.1.1	Up regulated	684	Solyc04g054256.1.1.1	Up regulated			
620	Solyc07g052560.3.1.2	Up regulated	685	Solyc01g112120.4.1.1	Up regulated			
621	Solyc07g007420.3.1.3	Up regulated	686	Solyc02g067780.3.1.7	Up regulated			
622	Solyc07g054060.3.1.2	Up regulated	687	Solyc02g070600.3.1.1	Up regulated			
623	Solyc08g080270.3.1.1	Up regulated	688	Solyc02g067790.4.1.1	Up regulated			
624	Solyc05g024230.3.1.1	Up regulated	689	Solyc02g089780.3.1.1	Up regulated			
625	Solyc01g088260.3.1.1	Up regulated	690	Solyc02g094290.1.1.1	Up regulated			
626	Solyc08g082520.1.1.1	Up regulated	691	Solyc02g031730.1.1.1	Up regulated			
627	Solyc08g067300.1.1.1	Up regulated	692	Solyc03g115970.2.1.6	Up regulated			
628	Solyc08g007090.3.1.1	Up regulated	693	Solyc04g014290.1.1.1	Up regulated			
629	Solyc08g006330.3.1.1	Up regulated	694	Solyc04g014320.1.1.1	Up regulated			
630	Solyc08g068790.3.1.1	Up regulated	695	Solyc04g014330.1.1.1	Up regulated			
631	Solyc09g008830.3.1.2	Up regulated	696	Solyc04g014350.1.1.1	Up regulated			
632	Solyc09g092767.1.1.3	Up regulated	697	Solyc01g080230.3.1.8	Up regulated			
633	Solyc09g083090.4.1.1	Up regulated	698	Solyc05g006730.4.1.2	Up regulated			
634	Solyc01g103060.4.1.1	Up regulated	699	Solyc01g081020.1.1.1	Up regulated			
635	Solyc09g083040.1.1.1	Up regulated	700	Solyc05g012950.3.1.1	Up regulated			
636	Solyc10g150102.1.1.1	Up regulated	701	Solyc07g054240.2.1.1	Up regulated			
637	Solyc12g062690.2.1.3	Up regulated	702	Solyc07g063700.2.1.8	Up regulated			
638	Solyc03g119800.3.1.1	Up regulated	703	Solyc09g082700.2.1.1	Up regulated			
639	Solyc06g066710.1.1.1	Up regulated	704	Solyc09g092540.1.1.1	Up regulated			
640	Solyc07g038110.3.1.1	Up regulated	705	Solyc09g065750.3.1.1	Up regulated			
641	Solyc01g068520.1.1.1	Up regulated	706	Solyc09g097800.2.1.3	Up regulated			
642	Solyc10g006150.3.1.1	Up regulated	707	Solyc10g051120.3.1.4	Up regulated			
643	Solyc06g005460.1.1.1	Up regulated	708	Solyc11g069920.1.1.2	Up regulated			
644	Solyc07g062790.1.1.1	Up regulated	709	Solyc11g068560.2.1.1	Up regulated			
645	Solyc09g090685.1.1.3	Up regulated	710	Solyc04g018080.4.1.2	Up regulated			
646	Solyc04g017740.1.1.1	Up regulated	711	Solyc05g018870.3.1.1	Up regulated			
647	Solyc07g066360.1.1.1	Up regulated	712	Solyc06g005795.2.1.1	Up regulated			
648	Solyc03g082520.1.1.1	Up regulated	713	Solyc01g150172.1.1.16	Up regulated			
649	Solyc11g006450.3.1.2	Up regulated	714	Solyc01g090980.1.1.1	Up regulated			
650	Solyc12g088850.1.1.1	Up regulated	715	Solyc06g050870.3.1.1	Up regulated			

S.No.	id	Status	S.No.	id	Status	S.No.	id	Status
1	Solyc03g093390.4.1.1	Down regulated	66	Solyc01g080800.3.1.1	Down regulated	131	Solyc04g064920.4.1.1	Down regulated
2	Solyc10g052470.1.1.1	Down regulated	67	Solyc01g087800.2.1.1	Down regulated	132	Solyc04g078860.4.1.2	Down regulated
3	Solyc03g119910.4.1.2	Down regulated	68	Solyc01g091700.4.1.3	Down regulated	133	Solyc04g079470.3.1.1	Down regulated
4	Solyc07g052480.3.1.2	Down regulated	69	Solyc01g096880.4.1.2	Down regulated	134	Solyc04g074020.2.1.3	Down regulated
5	Solyc01g095140.4.1.2	Down regulated	70	Solyc01g097550.4.1.6	Down regulated	135	Solyc10g018290.2.1.1	Down regulated
6	Solyc08g079870.3.1.1	Down regulated	71	Solyc01g102340.3.1.3	Down regulated	136	Solyc05g006750.3.1.2	Down regulated
7	Solyc04g081870.4.1.1	Down regulated	72	Solyc01g103590.4.1.1	Down regulated	137	Solyc05g008960.3.1.4	Down regulated
8	Solyc03g006490.3.1.4	Down regulated	73	Solyc01g106640.2.1.1	Down regulated	138	Solyc05g012030.1.1.1	Down regulated
9	Solyc06g005465.1.1.3	Down regulated	74	Solyc01g106820.3.1.1	Down regulated	139	Solyc05g013410.3.1.1	Down regulated
10	Solyc04g058100.3.1.3	Down regulated	75	Solyc01g107520.3.1.1	Down regulated	140	Solyc05g015320.3.1.11	Down regulated
11	Solyc03g121540.3.1.19	Down regulated	76	Solyc01g109690.1.1.1	Down regulated	141	Solyc05g053860.4.1.1	Down regulated
12	Solyc10g086570.3.1.2	Down regulated	77	Solyc01g098060.1.1.1	Down regulated	142	Solyc05g053170.3.1.2	Down regulated
13	Solyc11g007880.1.1.1	Down regulated	78	Solyc01g098370.1.1.1	Down regulated	143	Solyc05g052890.3.1.5	Down regulated
14	Solyc07g009380.4.1.3	Down regulated	79	Solyc01g105960.2.1.3	Down regulated	144	Solyc05g047600.4.1.1	Down regulated
15	Solyc04g081530.1.1.1	Down regulated	80	Solyc01g108416.1.1.2	Down regulated	145	Solyc05g047590.4.1.2	Down regulated
16	Solyc04g009900.4.1.1	Down regulated	81	Solyc01g111010.4.1.3	Down regulated	146	Solyc06g006110.3.1.11	Down regulated
17	Solyc01g109150.4.1.1	Down regulated	82	Solyc02g022850.1.1.1	Down regulated	147	Solyc06g008930.3.1.2	Down regulated
18	Solyc12g008650.2.1.6	Down regulated	83	Solyc02g093670.3.1.2	Down regulated	148	Solyc06g035760.4.1.1	Down regulated
19	Solyc11g020330.1.1.1	Down regulated	84	Solyc02g093480.4.1.1	Down regulated	149	Solyc06g035700.1.1.1	Down regulated
20	Solyc07g047850.3.1.1	Down regulated	85	Solyc02g086452.1.1.2	Down regulated	150	Solyc06g034370.1.1.1	Down regulated
21	Solyc07g061730.3.1.1	Down regulated	86	Solyc02g083690.2.1.4	Down regulated	151	Solyc06g050800.4.1.4	Down regulated
22	Solyc08g014000.3.1.1	Down regulated	87	Solyc02g081030.4.1.1	Down regulated	152	Solyc06g054570.1.1.1	Down regulated
23	Solyc07g007750.3.1.1	Down regulated	88	Solyc02g080120.3.1.3	Down regulated	153	Solyc06g073580.4.1.3	Down regulated
24	Solyc06g074730.4.1.1	Down regulated	89	Solyc02g079980.3.1.4	Down regulated	154	Solyc06g072670.3.1.1	Down regulated
25	Solyc10g007960.1.1.1	Down regulated	90	Solyc02g079460.1.1.1	Down regulated	155	Solyc06g072460.1.1.1	Down regulated
26	Solyc03g117250.4.1.2	Down regulated	91	Solyc02g078500.2.1.4	Down regulated	156	Solyc06g072350.3.1.3	Down regulated
27	Solyc06g008590.3.1.1	Down regulated	92	Solyc02g077430.4.1.1	Down regulated	157	Solyc06g069640.3.1.1	Down regulated
28	Solyc10g084120.2.1.4	Down regulated	93	Solyc02g077400.4.1.1	Down regulated	158	Solyc06g068040.3.1.9	Down regulated
29	Solyc08g008045.1.1.1	Down regulated	94	Solyc02g077110.3.1.2	Down regulated	159	Solyc06g067870.3.1.3	Down regulated
30	Solyc03g006550.4.1.10	Down regulated	95	Solyc02g070180.3.1.1	Down regulated	160	Solyc06g063050.4.1.3	Down regulated
31	Solyc03g006545.1.1.1	Down regulated	96	Solyc02g067380.4.1.1	Down regulated	161	Solyc06g063040.1.1.1	Down regulated
32	Solyc03g096290.3.1.1	Down regulated	97	Solyc02g064800.3.1.6	Down regulated	162	Solyc06g062450.3.1.3	Down regulated
33	Solyc12g006140.2.1.1	Down regulated	98	Solyc02g090450.3.1.4	Down regulated	163	Solyc06g062390.3.1.3	Down regulated
34	Solyc01g105050.3.1.2	Down regulated	99	Solyc02g083920.1.1.1	Down regulated	164	Solyc06g060640.1.1.1	Down regulated
35	Solyc08g074683.1.1.1	Down regulated	100	Solyc03g006452.1.1.1	Down regulated	165	Solyc06g076910.3.1.2	Down regulated
36	Solyc03g093790.3.1.1	Down regulated	101	Solyc03g007940.4.1.3	Down regulated	166	Solyc09g018970.1.1.1	Down regulated
37	Solyc05g054050.4.1.1	Down regulated	102	Solyc03g025460.3.1.1	Down regulated	167	Solyc06g069740.1.1.1	Down regulated
38	Solyc08g061920.3.1.2	Down regulated	103	Solyc03g031530.4.1.1	Down regulated	168	Solyc06g069600.3.1.5	Down regulated
39	Solyc10g047810.3.1.1	Down regulated	104	Solyc03g034000.3.1.3	Down regulated	169	Solyc06g063200.2.1.2	Down regulated
40	Solyc10g083300.2.1.4	Down regulated	105	Solyc03g034100.4.1.2	Down regulated	170	Solyc06g084190.3.1.1	Down regulated
41	Solyc09g009540.4.1.1	Down regulated	106	Solyc03g058440.1.1.1	Down regulated	171	Solyc06g084040.3.1.1	Down regulated
42	Solyc02g070980.1.1.1	Down regulated	107	Solyc03g083730.1.1.1	Down regulated	172	Solyc07g005680.4.1.6	Down regulated
43	Solyc03g115900.4.1.2	Down regulated	108	Solyc03g082350.2.1.2	Down regulated	173	Solyc07g006310.1.1.1	Down regulated
44	Solyc08g067320.3.1.1	Down regulated	109	Solyc03g080100.4.1.5	Down regulated	174	Solyc07g008600.1.1.1	Down regulated
45	Solyc03g005770.3.1.1	Down regulated	110	Solyc03g078620.1.1.1	Down regulated	175	Solyc07g009060.4.1.1	Down regulated
46	Solyc03g005780.3.1.1	Down regulated	111	Solyc03g095980.3.1.1	Down regulated	176	Solyc07g009170.4.1.9	Down regulated
47	Solyc08g079860.2.1.1	Down regulated	112	Solyc03g122080.3.1.5	Down regulated	177	Solyc07g016215.1.1.1	Down regulated
48	Solyc06g069730.3.1.2	Down regulated	113	Solyc03g121240.1.1.1	Down regulated	178	Solyc08g068480.1.1.1	Down regulated
49	Solyc06g035490.3.1.1	Down regulated	114	Solyc03g115040.4.1.2	Down regulated	179	Solyc07g043230.3.1.3	Down regulated
50	Solyc02g071000.1.1.1	Down regulated	115	Solyc03g114530.4.1.1	Down regulated	180	Solyc07g066560.1.1.1	Down regulated
51	Solyc04g011880.1.1.1	Down regulated	116	Solyc03g111710.3.1.5	Down regulated	181	Solyc07g065500.2.1.1	Down regulated
52	Solyc10g007600.3.1.1	Down regulated	117	Solyc03g111290.2.1.1	Down regulated	182	Solyc07g064760.2.1.2	Down regulated
53	Solyc02g070990.1.1.1	Down regulated	118	Solyc03g119660.2.1.5	Down regulated	183	Solyc07g064720.3.1.5	Down regulated
54	Solyc02g071010.1.1.1	Down regulated	119	Solyc04g011870.1.1.1	Down regulated	184	Solyc07g063600.3.1.1	Down regulated
55	Solyc02g070940.1.1.1	Down regulated	120	Solyc04g011860.1.1.1	Down regulated	185	Solyc07g062700.3.1.7	Down regulated
56	Solyc02g070970.1.1.1	Down regulated	121	Solyc04g007940.4.1.2	Down regulated	186	Solyc07g062040.3.1.4	Down regulated
57	Solyc04g007690.3.1.6	Down regulated	122	Solyc04g007760.3.1.2	Down regulated	187	Solyc07g061890.1.1.1	Down regulated
58	Solyc01g006550.3.1.1	Down regulated	123	Solyc04g007750.4.1.2	Down regulated	188	Solyc08g006100.4.1.2	Down regulated
59	Solyc01g017600.3.1.1	Down regulated	124	Solyc04g005660.4.1.2	Down regulated	189	Solyc08g007150.1.1.1	Down regulated
60	Solyc01g009690.3.1.1	Down regulated	125	Solyc04g005480.1.1.1	Down regulated	190	Solyc08g007430.2.1.5	Down regulated
61	Solyc01g006310.3.1.4	Down regulated	126	Solyc04g016190.1.1.1	Down regulated	191	Solyc08g007830.1.1.1	Down regulated
62	Solyc01g006520.3.1.2	Down regulated	127	Solyc04g026020.3.1.1	Down regulated	192	Solyc08g008305.1.1.1	Down regulated
63	Solyc01g067460.3.1.1	Down regulated	128	Solyc04g053030.1.1.1	Down regulated	193	Solyc08g007840.3.1.2	Down regulated
64	Solyc01g079830.3.1.1	Down regulated	129	Solyc04g051360.3.1.2	Down regulated	194	Solyc08g014490.1.1.1	Down regulated
65	Solyc01g080680.3.1.16	Down regulated	130	Solyc04g051270.2.1.1	Down regulated	195	Solyc08g036620.4.1.3	Down regulated

S.No.	id	Status	S.No.	id	Status	S.No.	id	Status
196	Solyc08g036640.3.1.3	Down regulated	261	Solyc12g017870.2.1.1	Down regulated	326	Solyc08g068110.1.1.4	Down regulated
197	Solyc08g065430.3.1.1	Down regulated	262	Solyc12g019550.2.1.2	Down regulated	327	Solyc08g080520.4.1.26	Down regulated
198	Solyc08g067550.1.1.1	Down regulated	263	Solyc12g044380.3.1.1	Down regulated	328	Solyc08g077490.2.1.1	Down regulated
199	Solyc08g078840.3.1.4	Down regulated	264	Solyc12g049190.3.1.1	Down regulated	329	Solyc09g092760.2.1.2	Down regulated
200	Solyc08g074510.1.1.2	Down regulated	265	Solyc12g043150.3.1.1	Down regulated	330	Solyc10g051070.1.1.2	Down regulated
201	Solyc09g008913.1.1.2	Down regulated	266	Solyc12g049500.3.1.1	Down regulated	331	Solyc10g085700.1.1.2	Down regulated
202	Solyc09g098620.2.1.3	Down regulated	267	Solyc12g057070.2.1.2	Down regulated	332	Solyc12g013680.3.1.1	Down regulated
203	Solyc09g005500.3.1.1	Down regulated	268	Solyc12g056460.3.1.2	Down regulated	333	Solyc12g013730.3.1.1	Down regulated
204	Solyc09g014720.3.1.1	Down regulated	269	Solyc12g056270.2.1.1	Down regulated	334	Solyc12g087930.1.1.1	Down regulated
205	Solyc06g068815.1.1.1	Down regulated	270	Solyc12g055700.3.1.3	Down regulated	335	Solyc02g087860.3.1.2	Down regulated
206	Solyc09g074590.1.1.1	Down regulated	271	Solyc12g089330.2.1.3	Down regulated	336	Solyc02g076630.1.1.1	Down regulated
207	Solyc09g072690.1.1.1	Down regulated	272	Solyc12g088940.3.1.10	Down regulated	337	Solyc03g116520.1.1.1	Down regulated
208	Solyc09g082760.3.1.2	Down regulated	273	Solyc12g100030.2.1.1	Down regulated	338	Solyc04g063245.1.1.1	Down regulated
209	Solyc09g092310.1.1.1	Down regulated	274	Solyc12g098540.2.1.9	Down regulated	339	Solyc04g078370.3.1.3	Down regulated
210	Solyc09g092330.3.1.1	Down regulated	275	Solyc12g096630.2.1.6	Down regulated	340	Solyc05g011840.3.1.3	Down regulated
211	Solyc09g092600.3.1.1	Down regulated	276	Solyc12g099870.3.1.1	Down regulated	341	Solyc05g005340.3.1.2	Down regulated
212	Solyc09g098425.1.1.1	Down regulated	277	Solyc03g008015.1.1.2	Down regulated	342	Solyc05g021580.3.1.3	Down regulated
213	Solyc10g009150.3.1.2	Down regulated	278	Solyc05g018403.1.1.1	Down regulated	343	Solyc06g076020.3.1.1	Down regulated
214	Solyc10g008440.3.1.4	Down regulated	279	Solyc08g006765.1.1.2	Down regulated	344	Solyc07g150147.1.1.1	Down regulated
215	Solyc10g008270.3.1.2	Down regulated	280	Solyc11g071580.2.1.2	Down regulated	345	Solyc07g053340.2.1.2	Down regulated
216	Solyc10g008120.4.1.1	Down regulated	281	Solyc01g099480.4.1.1	Down regulated	346	Solyc07g009070.4.1.1	Down regulated
217	Solyc10g006530.4.1.1	Down regulated	282	Solyc03g110990.1.1.1	Down regulated	347	Solyc07g009080.4.1.1	Down regulated
218	Solyc10g005400.3.1.1	Down regulated	283	Solyc09g065350.1.1.1	Down regulated	348	Solyc01g110770.2.1.3	Down regulated
219	Solyc05g015790.2.1.1	Down regulated	284	Solyc11g006150.1.1.2	Down regulated	349	Solyc08g067330.1.1.1	Down regulated
220	Solyc10g009310.4.1.2	Down regulated	285	Solyc01g110630.4.1.1	Down regulated	350	Solyc09g097770.3.1.2	Down regulated
221	Solyc10g008710.3.1.2	Down regulated	286	Solyc04g009120.3.1.1	Down regulated	351	Solyc03g007930.4.1.1	Down regulated
222	Solyc10g017960.2.1.1	Down regulated	287	Solyc02g150128.1.1.1	Down regulated	352	Solyc09g072770.1.1.1	Down regulated
223	Solyc10g052550.1.1.1	Down regulated	288	Solyc02g085770.4.1.4	Down regulated	353	Solyc05g010530.3.1.1	Down regulated
224	Solyc10g044700.2.1.1	Down regulated	289	Solyc01g009930.3.1.1	Down regulated	354	Solyc02g063400.3.1.1	Down regulated
225	Solyc10g044680.2.1.3	Down regulated	290	Solyc02g068415.1.1.1	Down regulated	355	Solyc12g005540.1.1.1	Down regulated
226	Solyc10g076570.2.1.1	Down regulated	291	Solyc01g014140.3.1.1	Down regulated	356	Solyc12g005550.3.1.2	Down regulated
227	Solyc10g078720.2.1.7	Down regulated	292	Solyc08g082380.1.1.1	Down regulated	357	Solyc02g067600.1.1.1	Down regulated
228	Solyc10g079110.2.1.1	Down regulated	293	Solyc10g050510.1.1.1	Down regulated	358	Solyc07g009040.3.1.1	Down regulated
229	Solyc10g080690.2.1.1	Down regulated	294	Solyc02g069300.1.1.1	Down regulated	359	Solyc01g108710.3.1.1	Down regulated
230	Solyc10g081570.3.1.1	Down regulated	295	Solyc10g079300.2.1.5	Down regulated	360	Solyc07g039343.1.1.4	Down regulated
231	Solyc10g083320.3.1.1	Down regulated	296	Solyc10g079590.1.1.3	Down regulated	361	Solyc06g065073.1.1.1	Down regulated
232	Solyc10g083880.2.1.2	Down regulated	297	Solyc10g080640.3.1.8	Down regulated	362	Solyc03g119670.3.1.4	Down regulated
233	Solyc10g083940.1.1.1	Down regulated	298	Solyc10g085420.3.1.2	Down regulated	363	Solyc05g053180.3.1.2	Down regulated
234	Solyc10g079630.1.1.2	Down regulated	299	Solyc11g017130.2.1.3	Down regulated	364	Solyc07g044970.1.1.1	Down regulated
235	Solyc10g079790.1.1.2	Down regulated	300	Solyc12g006600.3.1.1	Down regulated	365	Solyc11g073238.1.1.4	Down regulated
236	Solyc11g005270.1.1.1	Down regulated	301	Solyc12g009730.3.1.1	Down regulated	366	Solyc11g005240.1.1.1	Down regulated
237	Solyc11g005280.1.1.1	Down regulated	302	Solyc12g009780.1.1.3	Down regulated	367	Solyc01g097710.1.1.1	Down regulated
238	Solyc11g005630.1.1.1	Down regulated	303	Solyc12g096620.1.1.1	Down regulated	368	Solyc11g072900.1.1.1	Down regulated
239	Solyc11g007220.2.1.1	Down regulated	304	Solyc01g110000.3.1.1	Down regulated	369	Solyc01g110720.2.1.1	Down regulated
240	Solyc11g007250.2.1.1	Down regulated	305	Solyc02g092460.3.1.5	Down regulated	370	Solyc01g110930.1.1.1	Down regulated
241	Solyc11g007370.3.1.1	Down regulated	306	Solyc02g086760.1.1.1	Down regulated	371	Solyc02g089700.4.1.3	Down regulated
242	Solyc11g007890.2.1.4	Down regulated	307	Solyc02g085760.2.1.2	Down regulated	372	Solyc03g006230.1.1.1	Down regulated
243	Solyc11g010360.3.1.1	Down regulated	308	Solyc02g084130.4.1.4	Down regulated	373	Solyc04g011990.3.1.1	Down regulated
244	Solyc11g010440.3.1.1	Down regulated	309	Solyc02g068670.3.1.5	Down regulated	374	Solyc04g053020.2.1.1	Down regulated
245	Solyc11g011630.3.1.1	Down regulated	310	Solyc02g072220.1.1.1	Down regulated	375	Solyc04g078325.1.1.1	Down regulated
246	Solyc11g011640.1.1.1	Down regulated	311	Solyc03g033860.1.1.1	Down regulated	376	Solyc04g079450.4.1.2	Down regulated
247	Solyc11g011660.1.1.1	Down regulated	312	Solyc03g120800.4.1.1	Down regulated	377	Solyc04g081720.2.1.1	Down regulated
248	Solyc11g011710.1.1.1	Down regulated	313	Solyc03g114740.4.1.4	Down regulated	378	Solyc05g055650.3.1.2	Down regulated
249	Solyc11g018800.3.1.1	Down regulated	314	Solyc04g008470.3.1.2	Down regulated	379	Solyc05g054470.3.1.3	Down regulated
250	Solyc11g005290.1.1.1	Down regulated	315	Solyc04g074050.4.1.2	Down regulated	380	Solyc07g052220.1.1.1	Down regulated
251	Solyc11g068620.2.1.1	Down regulated	316	Solyc05g008910.3.1.2	Down regulated	381	Solyc08g069180.3.1.2	Down regulated
252	Solyc11g071740.2.1.1	Down regulated	317	Solyc05g014000.4.1.4	Down regulated	382	Solyc08g079970.2.1.1	Down regulated
253	Solyc11g072980.1.1.1	Down regulated	318	Solyc05g056520.4.1.1	Down regulated	383	Solyc08g068130.1.1.1	Down regulated
254	Solyc11g067250.3.1.2	Down regulated	319	Solyc06g009190.4.1.4	Down regulated	384	Solyc10g078170.3.1.1	Down regulated
255	Solyc12g006997.1.1.1	Down regulated	320	Solyc01g073780.2.1.1	Down regulated	385	Solyc06g007910.4.1.2	Down regulated
256	Solyc12g007240.3.1.1	Down regulated	321	Solyc06g048740.3.1.1	Down regulated	386	Solyc10g076260.2.1.1	Down regulated
257	Solyc12g009800.3.1.1	Down regulated	322	Solyc06g048735.1.1.1	Down regulated	387	Solyc11g018774.1.1.1	Down regulated
258	Solyc12g013700.2.1.2	Down regulated	323	Solyc08g007820.1.1.1	Down regulated	388	Solyc12g005520.1.1.1	Down regulated
259	Solyc12g013710.2.1.3	Down regulated	324	Solyc08g036505.2.1.1	Down regulated	389	Solyc01g073840.1.1.1	Down regulated
260	Solyc12g017460.1.1.1	Down regulated	325	Solyc08g067690.2.1.1	Down regulated	390	Solyc02g067770.3.1.1	Down regulated

S.No.	id	Status
391	Solyc02g072450.4.1.2	Down regulated
392	Solyc00g500353.1.1.2	Down regulated
393	Solyc04g011820.1.1.1	Down regulated
394	Solyc04g079460.1.1.1	Down regulated
395	Solyc04g011960.2.1.1	Down regulated
396	Solyc01g079930.3.1.2	Down regulated
397	Solyc05g012430.1.1.2	Down regulated
398	Solyc05g018310.1.1.1	Down regulated
399	Solyc07g006480.4.1.1	Down regulated
400	Solyc07g052790.3.1.4	Down regulated
401	Solyc08g068140.4.1.1	Down regulated
402	Solyc08g016270.3.1.1	Down regulated
403	Solyc02g033030.3.1.1	Down regulated
404	Solyc01g014320.4.1.1	Down regulated
405	Solyc12g009510.1.1.2	Down regulated
406	Solyc12g009550.2.1.1	Down regulated
407	Solyc01g067160.4.1.2	Down regulated
408	Solyc12g009720.3.1.1	Down regulated
409	Solyc04g081790.3.1.4	Down regulated
410	Solyc06g083480.4.1.3	Down regulated
411	Solyc06g007720.2.1.2	Down regulated
412	Solyc09g098385.1.1.2	Down regulated
413	Solyc02g079240.1.1.1	Down regulated

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Summary of my publications and attended conferences/workshops

First author publications

- **Srivastava, R.,** Sirohi, P., Chauhan, H. and Kumar, R. (2021) The enhanced phosphorus use efficiency in phosphate-deficient and mycorrhiza-inoculated barley seedlings involves activation of different sets of PHT1 transporters in roots. Planta. 254 (1-17).
- **Srivastava, R.,** Basu, S. and Kumar, R. (2021) Phosphorus starvation response dynamics and management in plants for sustainable agriculture. Journal of Plant Biochemistry and Biotechnology. 30 (829-847).
- **Srivastava, R.,** Roychowdhury, A. and Kumar, R. (2022) Host SPX-PHR regulatory circuit: the molecular dynamo steering mycorrhization in plants. Plant Cell Reports. 41 (1329-1332).
- **Srivastava, R.,** Akash., Parida, A.P., Chauhan, P.K., Kumar, R. (2020) Identification, structure analysis, and transcript profiling of purple acid phosphatases under Pi deficiency in tomato (*Solanum lycopersicum*.) and its wild relatives. Int. J. Biol. Macromol. 165 (2253-2266).
- **Srivastava, R.** and Kumar, R. (2019) The expanding roles of APETALA2/Ethylene Responsive Factors and their potential applications in crop improvement. Briefings in functional genomics. 18 (240-254).

Second author publication & book chapter

- Roychowdhury, A., **Srivastava, R.,** Akash, Shukla, G., Zehirov, G., Mishev, K. and Kumar, R., 2023. Metabolic footprints in phosphate-starved plants. Physiology and Molecular Biology of Plants, pp.1-13.
- Akash, **Srivastava R** and Kumar R*. VIGS-based gene silencing for assessing mineral nutrient acquisition in a book entitled 'Plant Gene Silencing II' part of the Methods in Molecular Biology series, published by Humana Press, USA, eds Mysore Kirankumar and Muthappa Senthil-Kumar, Springer publishing group *corresponding author. (165-179).

Equal first author contribution

- Singh, N.R.R., Roychowdhury, A., **Srivastava, R.,** Gaganan, G.A., Parida, A.P. and

Kumar, R., (2023). Silencing of SlSPX1 and SlSPX2 promote growth and root mycorrhization in tomato (Solanum lycopersicum L.) seedlings. Plant Science, 333, p.111723.

Equal contributions first author publications

- An integrated omics approach reveals sucrose-dependent role of tomato PAP26b in Pi acquisition in tomato: Akash1a#, **Rajat Srivastava1a#**, Kapil Sharma, and Rahul Kumar1 (# equal contribution).

Poster presentations and workshop attended

- Presented poster on "Understanding the SPX-PHR controlled mycorrhizal colonization in tomato roots using Virus-induced gene silencing approach" at International Conference on Virus Evolution, Infection and Disease Control (ICVEIDC-2022) by department of Biotechnology & Bioinformatics, School of Life Sciences, University of Hyderabad, India-500046.
- Presented poster on "Understanding the genetic regulatory mechanism controlling PUE and PAE upon mycorrhizal colonization in tomato", in 12th India-Japan Science and Technology. International conference on Frontier areas of science and technology (ICFAST-2022) held at University of Hyderabad, Hyderabad 500046, INDIA during september 09-10-2022.
- Participated in SERB sponsored workshop "Hands on training on CRISPR/Cas9 mediated gene editing in plants" organized by department of Plant Sciences, University of Hyderabad on October 3-10, 2021.
- Participated in "National conference on Frontiers in Plant Biology", 31st January - 1st February 2020, organized by department of Plant Sciences, School of Life Sciences, university of Hyderabad, 500046, India-500046.
- Presented poster on "Transcriptomics and functional genomics for improving phosphate use efficiency in tomato (Solanum lycopersicum)" at National conference on Integrative Plant Biochemistry and Biotechnology, 08-09 November 2019, organized by Society for Plant Biochemistry and Biotechnology, New Delhi and IICAR-IRR, Hyderabad India-500046.
- Presented poster on "Transcriptomics and functional genomics for improving phosphorous use efficiency in tomato" (*Solanum Lycopersicum L*), at 4th International

conference on agriculture and animal husbandry "Advances in agriculture and animal sciences towards global food security" organized in association with Life science society of Hyderabad during August 28-30,2019 at University of Hyderabad.

- Participated in the 59th Annual Conference of Association of Microbiologist of India (AMI-2018) and International Symposium on Host Pathogen Interactions held on 9th-12th December 2018 by School of Life Sciences, university of Hyderabad, Hyderabad, India-500046.
- Presented the poster entitled "Understanding the molecular circuitry underlying low phosphorous response in tomato" in BioQuest-2017 held on 12th and 13th October 2017 at School of Life Sciences, University of Hyderabad, Hyderabad, India-500046.
- Participant in the Nano3Bio dissemination event entitled "The future of Chitosans" held on 20th September 2017, organized by dept. of plant Sciences, School of Life Sciences, university of Hyderabad, Hyderabad, India-500046.
- Attended one day "Workshop on Mendeley" held at University of Hyderabad on 9th March 2017, organized by IGM Library in collaboration with Elsevier Publisher.
- Participant in online workshop on "Hands-On Training on CRISPR/Cas9 Mediated Gene-editing in Plants" 3-10 October, 2021 by SERB-SSR (CRG/2018/001033) at Department of Plant Sciences, University of Hyderabad.

"Screening of tomato germplasm and functional characterization of candidate purple acid phosphatase genes under phosphate deficiency "

by Rajat Srivastava

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